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THE RHIZOSPHERE AND PLANT GROWTH



Beltsville Symposium XIV

May 8-11, 1989

Program and Abstract Booklet

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Department of
Agriculture**



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Beltsville Symposium XIV

THE RHIZOSPHERE AND PLANT GROWTH

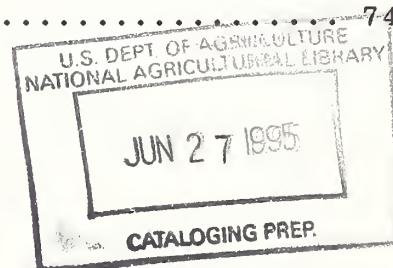
May 8-11, 1989

Beltsville Agricultural Research Center
Agricultural Research Service
U. S. Department of Agriculture
Beltsville, Maryland

INFORMATION, PROGRAM, AND ABSTRACTS

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SCHEDULE OF EVENTS

SUNDAY, MAY 7	Holiday Inn-College Park
4:00- 8:00 p.m.	Registration - The Maryland Room
6:30	Reception
MONDAY, MAY 8	Auditorium - Main Building
8:20 a.m.	Beltsville Agricultural Research Center
8:40	Welcome and Introduction
9:25-12:10	Keynote Address: Dr. Albert Rovira
12:10- 3:00 p.m.	SESSION I: The Rhizosphere: General Aspects Lunch - POSTER SESSION A, Setup and Viewing Grand Ballroom, Holiday Inn
1:30- 2:30	Commemorative Ceremony for Henry A. Wallace
2:30- 3:00	Refreshments
3:00- 4:45	SESSION I (Continued)
7:30- 9:30	SESSION II: Methods for Rhizosphere Studies
9:00-11:00	POSTER SESSION A (Continued) - Social Hours Grand Ballroom - Holiday Inn
TUESDAY, MAY 9	
8:10-12:10 a.m.	SESSION III: Plant-Microbe Interactions
12:10- 1:40 p.m.	Lunch - Set Up for POSTER SESSION B BARC Tour 1 *
1:40- 3:40	SESSION III (Continued)
3:40	Refreshments
3:40- 4:40	BARC Tour 2 *
4:00- 6:00	POSTER SESSION B
9:00-11:00	POSTER SESSION B (Continued) - Social Hours Grand Ballroom - Holiday Inn
WEDNESDAY, MAY 10	
8:10-12:10 a.m.	SESSION IV: Rhizosphere Interactions and Plant Pest Control
12:10- 1:30 p.m.	Lunch Break Remove Posters
1:40- 4:30	SESSION IV (Continued)
6:00	Social Hour Grand Ballroom - Holiday Inn
7:00	Banquet (Ticket Required)
8:30	FAR-B Awards
8:45	Speaker: Dr. Alan Hecht, Director National Climate Program, NOAA
THURSDAY, MAY 11	
8:10-12:30 a.m.	SESSION V: Plant Growth Promotion
12:30- 2:00 p.m.	Lunch
2:00- 3:30	Informal Roundtable Discussion
2:00	BARC Tour 3 *
2:00	National Arboretum Tour

* Information and sign-up at registration table.

SYMPOSIUM ORGANIZATION

Beltsville Agricultural Research Center (BARC)
Edward B. Knippling, Director, Beltsville Area
K. Darwin Murrell, Associate Area Director

Symposium Chairman
Donald L. Keister

Symposium Secretary
Margaret A. Blackwell

Program Committee
George C. Papavizas, Co-chairman, Biocontrol of Plant Diseases
Laboratory
Donald L. Keister, Co-chairman, Nitrogen Fixation and Soybean
Genetics Laboratory
Donald D. Kaufman, Soil Microbial Systems Laboratory
Robert E. Davis, Microbiology and Plant Pathology Laboratory
Daniel R. Shelton, Pesticide Degradation Laboratory

Local Arrangements Committee
Robert C. Leffel, Chairman
Lawrence J. Sikora, Banquet Arrangements
Daniel R. Shelton, Post-Conference Tours
Amanda M. Bolgiano, Local Transportation
Jack J. Meisinger, Poster Sessions
Gordon T. Carpenter, Sr., Audiovisuals, Auditorium Coordinator

Publicity
Deborah R. Fravel

Publications
Perry B. Cregan
Donald L. Keister

Finance
Frank J. Longen, FAR-B

SYMPOSIUM PROCEEDINGS

The proceedings of the Symposium will be published jointly in hard cover as Volume XIV of the Beltsville series and as a special volume of the journal, "Plant and Soil". The publisher will be Kluwer Academic Publishers.

All oral presentations will be published. In addition, a one-page summary of all poster presentations will be included. The volume will be edited by D. L. Keister and P. B. Cregan. All speakers and registrants who have paid the full registration fee will receive a copy of the hardbound publication. Others may order the volume, at a pre-publication price of \$65.00 (33% discount). Order forms can be obtained from the Conference Secretary at the Registration Desk.

ACKNOWLEDGMENTS

The Symposium is co-sponsored by Friends of Agricultural Research-Beltsville, Inc. (FAR-B). FAR-B is a non-profit organization dedicated to supporting the research and education programs which have been maintained for over 75 years at the Beltsville Agricultural Research Center (BARC). Voting members are individuals and groups who want to help this world-renowned research center for the agricultural sciences continue in excellence.

If you would like to join FAR-B as a member or sustaining member and help support their efforts, please write to FAR-B, P.O. Box 1061, Beltsville, MD 20705-1061.

The following sponsors have contributed to the ARS or to FAR-B to help support the symposium:

A. Daigger & Co., Wheeling, Illinois

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FMC Corporation, Chemical Research and Development Center, Princeton, New Jersey

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Shimadzu Scientific Instruments, Inc., Columbia, Maryland

Flowers are courtesy of the Florist and Nursery Crops Laboratory, Roger A. Lawson, Research Leader.

1990 Beltsville Symposium: "Remote Sensing for Agriculture"

Remote sensing is a method for obtaining rapid, cost effective information concerning the status of agriculture, both in the United States and abroad. Several operational agencies of the U.S. Department of Agriculture utilize satellite data to augment traditional sources of information, while aerial photography has been used for many years. This information is used in regularly issued commodity assessments.

The 1990 Symposium, to be sponsored jointly by the USDA-ARS, Beltsville Agricultural Research Center and the NASA Goddard Space Flight Center, Greenbelt, MD, will address current research in remote sensing, emphasizing our increasing capability to monitor vegetation status, new applications in hydrology, and the development of improved sensor technologies.

Session Chairman: John C. Price (301) 344-2688
Remote Sensing Research Laboratory
USDA, ARS
Room 338, Building 001, BARC-West
Beltsville, Maryland 20705

1991 Beltsville Symposium: "Plant Photomorphogenesis"

The photoregulation of plant growth and development encompasses many phenomena including seed germination, chloroplast development, root and shoot growth, leaf expansion, distribution of photosynthetic products, flowering, fruit ripening, and leaf abscission, all of significance to crop quality and productivity. With the support of emerging new research technologies in photochemistry, immunochemistry, and molecular genetics, research on phytochrome and photomorphogenesis has undergone an acceleration of activity and progress.

The year 1991 will mark the 40th anniversary of the beginning of phytochrome research. In 1951, Beltsville scientists discovered the phenomenon of red - far red reversibility of photoresponses and predicted the existence of a photoreversible photoreceptor. Approximately two decades of work at Beltsville by many American and foreign investigators led ultimately to the isolation of the pigment. In view of this anniversary it is proposed that the European Photomorphogenesis Group will join with the Beltsville Symposium at its 1991 meeting.

The 1991 Symposium will provide an opportunity to evaluate recent progress in photomorphogenesis research, to identify important, remaining and new questions, and to honor U.S. and foreign participants in the pioneering work at Beltsville. The symposium will include topics on blue light and UV photoperception, as well as phytochrome topics.

Session Chairman: William J. VanDerWoude (301) 344-3607
Plant Photobiology Lab., USDA, ARS
Building 046A
Beltsville Agricultural Research Center
Beltsville, Maryland 20705

BACKGROUND OF THE SYMPOSIUM

The annual Beltsville Symposium was established in 1976 to provide a forum for discussions among scientists involved in research that has potential for advancing agriculture and agricultural sciences. The fourteenth symposium in this series focuses on the rhizosphere and plant growth.

Much agricultural research in the past has treated the soil as a "black box" due to the difficulties in studying the complex interactions which take place in the rhizosphere. An empirical approach to plant growth served agriculture well as long as fertilizers, pesticides, water and land were abundant and inexpensive. However, we now recognize that the empirical approach is not sufficient as we consider how to maintain soil productivity and crop yields as well as environmental quality.

Improved crop management and crop protection systems are needed to promote sustainable agriculture and improve the quality of the environment. Cost-effective systems such as biological control and symbiotic nitrogen fixation, that would lessen the need for agricultural chemicals and decrease energy inputs, also would lessen environmental contamination and increase cost effectiveness. To design such systems the manipulation of soil microbes, both indigenous and introduced, is increasingly being advocated. Advances in plant and microbial molecular biology is offering possibilities for designing specific plant-microbe associations. Hopefully, this symposium will define the present state of rhizosphere research, emphasize current problems, explore potential solutions, and describe the potential for modern biotechnology applications to these problems. We believe that this is the first symposium in the U.S. that will bring together so many disciplines (microbiology, plant pathology, plant physiology, molecular biology, ecology and soil science) together under the theme of the plant rhizosphere.

Past Symposia:

- Virology in Agriculture - 1976
- Biosystematics in Agriculture - 1977
- Animal Reproduction - 1978
- Human Nutrition Research - 1979
- Biological Control in Crop Production - 1980
- Strategies of Plant Reproduction - 1981
- Genetic Engineering: Applications to Agriculture - 1982
- Agricultural Chemicals of the Future - 1983
- Frontiers of Membrane Research - 1984
- Biotechnology for Solving Agricultural Problems - 1985
- Research Instrumentation for the 21st Century - 1986
- Biomechanisms Regulating Growth and Development: Keys to Progress - 1987
- Biotic Diversity and Germplasm Preservation - Global Imperatives - 1988

Future Symposia:

- Remote Sensing for Agriculture - 1990
- Plant Photomorphogenesis - 1991



PROGRAM

BELTSVILLE SYMPOSIUM XIV: "THE RHIZOSPHERE AND PLANT GROWTH"
May 8-11, 1989SUNDAY REGISTRATION AND RECEPTION
May 74:00- 8:00 Registration-The Maryland Room-Holiday Inn
6:30 ReceptionMONDAY WELCOME AND INTRODUCTION
May 88:20 Donald L. Keister, Symposium Chairman
Edward B. Knippling, Director, Beltsville Area
R. Dean Plowman, Administrator, Agricultural Research Service

SESSION I: THE RHIZOSPHERE: GENERAL ASPECTS

Moderator - George C. Papavizas, Biocontrol of Plant Diseases
Laboratory, Beltsville Agricultural Research Center
(BARC)8:40- 9:25 (OR-01)* Keynote Address: Rhizosphere Research - 85 Years of
Progress and Frustration
Albert Rovira, CSIRO Division of
Soils, Adelaide, Australia9:25- 9:55 (OR-02) Substrate Flow in the Rhizosphere
James M. Lynch, Institute of Horticultural
Research, Littlehampton, West Sussex, U.K.10:00-10:30 (OR-03) Microbial Dynamics in the Rhizosphere
Glynn Bowen, International Atomic Energy Agency,
Vienna, Austria

10:35-11:05 Coffee Break

11:05-11:35 (OR-04) Plant Root Growth and Development
Richard W. Zobel, USDA-ARS, Ithaca, NY11:40-12:10 (OR-05) Root Colonization by Introduced and Indigenous
Microorganisms
Jennifer L. Parke, University of Wisconsin

12:15- 2:30 Lunch - POSTER SESSION A ** - Set-Up and Viewing

1:30- 2:30 Commemorative Ceremony for Henry A. Wallace
(Obtain copy of Program at Registration Desk)

2:30- 3:00 Refreshments

* Refers to Abstract Number: OR=Oral, PO=Poster

** See Poster Session A, page 12.

SESSION I (Continued)

Moderator - Daniel R. Shelton, Pesticide Degradation Laboratory, BARC

- 3:00- 3:30 (OR-06) Genetic Approaches for Studying Rhizosphere Colonization
Stephen T. Lam, CIBA-GEIGY Corp., NC

- 3:35- 4:05 (OR-07) Fate of Genes and Genetically Engineered Microorganisms in Soil and Rhizosphere
James M. Tiedje, Michigan State University

- 4:10- 4:40 (OR-08) Use of Mutants to Study the Impact of Chemoattraction to Sloughed Root Cap Cells on Pathogen Populations in the Rhizosphere
Martha C. Hawes, University of Arizona

MONDAY SESSION II: ASSESSMENT OF CURRENT METHODS AND INNOVATIVE NEW METHODS FOR RHIZOSPHERE STUDIES

EVENING
May 10 Moderator - Edwin L. Schmidt, University of Minnesota
7:30-9:30 pm

- (OR-09) Microbial Autecology in the Soil Rhizosphere
Edwin L. Schmidt, University of Minnesota

- (OR-10) Applications and Limitations to Rhizotrons and Minirhizotrons
Dan R. Upchurch, USDA-ARS, Lubbock, Texas

- (OR-11) Image Analyses of NMR and Video Recorded Root Systems
Alvin J. M. Smucker, Michigan State University

- (OR-12) The Development of a Conceptual Model of Root Growth
John H. Bowers, University of Florida and University of Wisconsin

- (OR-13) Conversion Factors to Use the Thymidine Method with Rhizosphere Bacteria
Henrik Christensen, University of Copenhagen, Denmark

9:00-11:00 pm POSTER SESSION A ** (continued)

Social Hours

** See Poster Session A, page 12.



TUESDAY SESSION III

May 9 PLANT-MICROBE INTERACTIONS: THE RHIZOBIUM-LEGUME SYMBIOSIS
Moderator - Robert C. Leffel, Nitrogen Fixation and Soybean Genetics Laboratory, BARC

- 8:10 Announcements
- 8:15- 8:45 (OR-14) Legume Inoculation - Successes and Failures
Robert H. Miller, North Carolina State University
- 8:50- 9:20 (OR-15) Importance of Saprophytic Competence for Introduced Microorganisms
Peter J. Bottomley, Oregon State University
- 9:25- 9:55 (OR-16) Enhancing the Colonization of Selected Bacteria in the Rhizosphere: The Rhizobium Experience
Martin Alexander, Cornell University
- 10:00-10:30 Coffee Break
- 10:30-11:00 (OR-17) Flavonoid Nodulation Signals Released by Alfalfa
Donald A. Phillips, University of California, Davis
- 11:05-11:35 (OR-18) Genetic Stability in Bradyrhizobium japonicum in the Field
Alan H. Gibson, CSIRO Division of Plant Industry, Canberra, Australia
- 11:40-12:10 (OR-19) Chemotaxis of Rhizobia to Root Phenolics that Induce Symbiotic Gene Expression
W. Dietz Bauer, Ohio State University
- 12:10- 1:40 Lunch - Set-up for POSTER SESSION B **

SESSION III (Continued)

Moderator - Deane F. Weber, Nitrogen Fixation and Soybean Genetics Laboratory

- 1:40- 2:10 (OR-20) Gene-for-Gene Interaction in the Legume-Rhizobium Symbiosis
Perry B. Cregan, Nitrogen Fixation and Soybean Genetics Laboratory, BARC
- 2:20- 2:40 (OR-21) Microbial Influence on Gene-for-Gene Interactions in Legume-Rhizobium Symbioses
Michael J. Sadowsky, Nitrogen Fixation and Soybean Genetics Laboratory, BARC
- 2:50- 3:20 (OR-22) Tripartite Interactions Between Legumes, Rhizobia and Mycorrhizal Fungi
Gabor J. Bethlenfalvay, USDA-ARS, Albany, CA

3:30- 4:00 Refreshments

4:00- 6:00 POSTER SESSION B **

9:00-11:00 POSTER SESSION B ** Social Hours-Grand Ballroom, Holiday Inn

** See Poster Session B, page 16.

WEDNESDAY SESSION IV

RHIZOSPHERE INTERACTIONS AND PLANT PEST CONTROL

Moderator - Robert D. Lumsden

Biocontrol of Plant Diseases Laboratory, BARC

8:10 Announcements

8:15- 8:45 (OR-23) Exudate Molecules Initiating Fungal Responses to Seeds and Roots
Eric B. Nelson, Cornell University

8:50- 9:20 (OR-24) Effects of Deleterious and HCN-Producing Pseudomonads
on Rhizosphere Interactions
Bob Schippers, University of Utrecht,
Baarn, The Netherlands

9:25- 9:50 (OR-25) Formulation and Delivery Systems of Biocontrol Agents
With Emphasis on Fungi
Jack A. Lewis, Biocontrol of Plant Diseases
Laboratory, BARC

9:55-10:25 Coffee Break

10:25-10:55 (OR-26) Formulation, Delivery Systems, and Marketing of Biocontrol Agents and Plant Growth Promoting Rhizobacteria
John L. McIntyre, Ecogen, Inc., Langhorne, PA

11:00-11:30 (OR-27) Induction of Rhizosphere Competence in the Biocontrol Fungus Trichoderma.
Ralph "Tex" Baker, Colorado State University

11:35-12:05 (OR-28) Mechanisms of Biocontrol of Soilborne Plant Pathogens With Rhizobacteria
Ilan Chet, The Hebrew University, Rehovot, Israel

12:10- 1:40 Lunch

SESSION IV (Continued)

Moderator - Milton N. Schroth, University of California, Berkeley

1:40- 2:10 (OR-29) Mechanisms of Biocontrol of Soilborne Plant Pathogens With Fungi
Deborah R. Fravel, Biocontrol of Plant Diseases Laboratory, BARC

2:10- 2:45 (OR-30) Identification and Regulation of Genes Involved in the Biocontrol of Soil-borne Plant Pathogens with Fluorescent Pseudomonads
Trevor N. Suslow, Advanced Genetic Science, Oakland, California

2:50- 3:20 (OR-31) Structure, Properties and Genetic Regulation of Siderophores
John B. Neilands, Univ. of California, Berkeley

3:25- 3:55 Refreshments

3:55- 4:25 (OR-32) Role of Antibiotics and Siderophores in Bacteria
Linda S. Thomashow, USDA-ARS, Pullman, WA

4:30- 5:00 (OR-33) Factors Influencing Siderophore-Mediated Biocontrol Activity of Rhizosphere Pseudomonas spp.
Joyce E. Loper, USDA-ARS, Corvallis, OR

WEDNESDAY EVENING

Grand Ballroom
Holiday Inn-College Park
10000 Baltimore Boulevard
College Park, MD 20740
301/345-6700

6:00 Social Hour

7:00 Banquet (Ticket required)

8:30 FAR-B Awards

8:45 Speaker: Dr. Alan Hecht
Director, National Climate Program
National Oceanic and Atmospheric Administration
"Climate Change: Science and Policy Implications"

- THURSDAY SESSION V
May 11 RHIZOSPHERE INTERACTIONS AND PLANT GROWTH PROMOTION
 Moderator - Donald D. Kaufman, Soil Microbial Systems
 Laboratory, BARC
- 8:10 Announcements
- 8:15- 8:40 (OR-34) Phytosiderophores: Their Existence, Structure and Function
 Volker Romheld, University of Hohenheim, FRG
- 8:45- 9:05 (OR-35) Rhizosphere Interactions and Siderophores
 Jeffrey S. Buyer, Soil Microbial Systems
 Laboratory, BARC
- 9:10- 9:35 (OR-36) Genetics of Iron Transport of Plant Growth-Promoting Pseudomonas putida
 John Leong, University of Utrecht, The Netherlands
- 9:40-10:05 (OR-37) Plant Growth Promotion Mediated by Bacterial Rhizosphere Colonizers
 Joe W. Kloepper, Allelix, Mississauga, Canada
 and Auburn University
- 10:10-10:35 Coffee Break
- 10:35-11:00 (OR-38) Microbial Production of Plant Hormones
 William T. Frankenberger, University of California, Riverside
- 11:05-11:25 (OR-39) VA Mycorrhizae: Their Presence, Role and Characterization
 Patricia D. Millner, Soil Microbial Systems
 Laboratory, BARC
- 11:30-11:55 (OR-40) Interactions of VA Mycorrhizae with Rhizobium, Azospirillum, Azotobacter and Phosphate Solubilizing Microbes
 J. M. Barea, Estazion Experimental del Azidin C
 Granada, Spain
- 12:00-12:25 (OR-41) Field Management of VA Mycorrhizal Fungi
 Lynette Abbott, University of Western Australia,
 Nedlands
- 12:30 Formal Closing of Symposium
- 2:00- 3:30 INFORMAL ROUNDTABLE DISCUSSION
 "Unanswered Issues"
 Moderator - James M. Lynch
- Panelists: Albert Rovira, Jennifer Parke, Martin Alexander,
James Tiedje, Joyce Loper, Ralph Baker,
Peter Bottomley



POSTER SESSION A
Monday, May 8, 1989

**Host-Rhizobium Interactions,
Rhizosphere Population Dynamics,
Root Growth and Biochemistry,
Microbes and Plant Nutrition**

Afternoon

12:30 - 1:30	Set-Up
1:30 - 3:00	Poster Viewing and Discussion
1:45 - 2:30	Authors of odd numbered posters (PO-01, PO-03, etc.) present.

Evening

9:00 -11:00	Poster Viewing and Discussion
9:00 -10:15	Authors of even numbered posters (PO-02, PO-04, etc.) present.

Please take down Monday Posters before 12:30, Tuesday.

<u>Poster No.</u>	<u>Title and Author</u>
PO-01	Extracellular Polysaccharide-Deficient Mutants of <u>Rhizobium</u> Strain CIAT899 Induce Chlorosis in Beans R. S. Araujo, G. A. Beattie, and J. Handelsman
PO-02	Rhizosphere Interaction Between Rhizobia and Nonlegumes G. L. Bender, J. Plazinski, and B. G. Rolfe
PO-03	Photosynthetic N ₂ -Fixing <u>Rhizobium</u> J. M. Ellis, M. Hungria, R. W. F. Hardy, and A. R. J. Eaglesham
PO-04	Physiological Comparisons Between Root and Stem Nodules of <u>Aeschynomene scabra</u> and <u>Sesbania rostrata</u> M. Hungria, A. R. J. Eaglesham, and R. W. F. Hardy
PO-05	Isolation and Characterization of Cowpea (<u>Vigna unguiculata</u>) Lectin R. Liu and E. L. Schmidt
PO-06	Distribution of P Metabolites and Compartmentation in Soybean Nodules as Studied by Electron Microscopy and ³¹ P NMR Spectroscopy D. Rolin, P. Pfeffer, J. Schmidt, R. Boswell, P. Cooke, and S. Jones
PO-07	Rapid Plate Assay for Hydrolytic Enzymes of <u>Rhizobium</u> N. Saleh-Rastin, M. A. Peterson, D. H. Hubbell and S. E. Coleman

<u>Poster No.</u>	<u>Title and Author</u>
PO-08	Evaluation of <u>Rhizobium meliloti</u> Strains Indigenous to Sonoran Desert Soils by Plasmid Profile and Intrinsic Antibiotic Resistance M. Shishido and I. L. Pepper
PO-09	Enhancing Rhizobial Colonization in the Rhizosphere of Legumes by a Systemic Fungicide M. A. Siddiqi and M. Alexander
PO-10	Levels of <u>nod</u> Gene Inducing Compounds in Uninoculated and Inoculated <u>Glycine max</u> (L.) Merr. cv. Bragg and Its Nodulation Mutants T. D. Sutherland, L. J. Schuller, B. B. Bassam, and P. M. Gresshoff
PO-11	Restriction Mapping and Subcloning of the Trifolitoxin Production and Resistance Genes from <u>Rhizobium leguminosarum</u> bv. <u>trifoli</u> T24 E. W. Triplett, M. J. Schink, and K. L. Noeldner
PO-12	The Beltsville Rhizobium Culture Collection P. van Berkum, R. F. Griffin, and D. F. Weber
PO-13	Competition for Nodulation of Soybean by <u>Bradyrhizobium japonicum</u> 123 and 138 in Missouri Soil R. E. Zdor and S. G. Pueppke
PO-20	Growth Rate of Rhizosphere Bacteria Measured by the Thymidine Method H. Christensen
PO-21	Chromosome and Symbiotic Plasmid Diversity Within a Naturally-Occurring Population of Clover Rhizobia D. H. Demezas, T. B. Reardon, J. M. Watson, and A. H. Gibson
PO-22	Stability of Antibiotic-Resistance Markers in <u>Bacillus cereus</u> UW85 L. J. Halverson and J. Handelsman
PO-23	Bacterial Characteristics Important to Rhizosphere Competence E. Hozore and M. Alexander
PO-24	Application of Hierarchical Theory to Microbial Faunal Interactions in Two Contrasting Soils N. G. Juma and P. M. Rutherford
PO-25	A Root Tip Enrichment Technique to Select Bacteria with High Root Colonizing Ability A. D. Rovira, D. M. Weller, and R. J. Cook
PO-26	Improved Rhizosphere Competence of <u>Trichoderma harzianum</u> by Protoplast Fusion A. Sivan and G. E. Harman

<u>Poster No.</u>	<u>Title and Author</u>
PO-27	Growth of Genetically-Altered <u>Pseudomonas solanacearum</u> in Soil and Rhizosphere J. W. Williamson, P. G. Hartel, and M. A. Schell
PO-28	Growth and Survival of Genetically-Altered <u>Pseudomonas aeruginosa</u> and <u>Pseudomonas putida</u> in Soil and in Rhizosphere K.-H. A. Yeung, M. A. Schell, and P. G. Hartel
PO-30	Photoregulation of Root:Shoot Ratio in Soybean Seedlings S. J. Britz
PO-31	Response of Symbiotic Soybeans to Acidified Soil G. R. Cline and K. Kaul
PO-32	The Occurrence of Cluster Roots in Actinorhizal Plants I. Louis, SRacette, and J. G. Torrey
PO-33	A Soil Biotron for Experimental Studies of Soil Biota J. Lussenhop and R. Fogel
PO-34	Water Uptake Patterns in Loblolly Pines as Seen With Magnetic Resonance Microscopy J. M. MacFall and G. A. Johnson
PO-35	Distribution of Assimilated Carbon Within the Plant and Rhizosphere of <u>Lolium perenne</u> : Comparison of Field and Laboratory Grown Plants A. A. Meharg and K. Killham
PO-36	Temperature Dependence of the Tonoplast and Plasma Membrane H ⁺ -ATPases from Maize Roots S-I. Tu and D. K. Brauer
PO-40	Characterization of Cultivar Specific Growth Promotion of Spring Wheat by <u>Bacillus</u> sp. C. P. Chanway and L. M. Nelson
PO-41	Effect of <u>Bacillus</u> strains on Growth of Pine (<u>Pinus contorta</u> Dougl.), Spruce (<u>Picea glauca</u> Voss.) and Douglas Fir (<u>Pseudotsuga menziesii</u> (Mirb.) Franco) C. P. Chanway, R. A. Radley, F. B. Holl, and P. A. Axelrood
PO-42	Rhizosphere Effect on Soil Organic Matter Decomposition W. Cheng and D. C. Coleman
PO-43	Interaction Between <u>Azospirillum</u> and the Host Plant: Model and Research Approaches M. Del Gallo
PO-44	Sulfur Oxidizing Microorganisms for Growth Promotion of Canola S. J. Grayston and J. J. Germida

Poster
No.

Title and Author

PO-45 Microbe Enhanced P Uptake by Corn Under No Till and
Conventional Till
H. M. Kunishi

PO-46 Influence of Nitrogen Fertilization on Photosynthate
Distribution and Utilization by Rhizosphere Microorganisms in
Wheat
E. Liljeroth, J. A. van Veen, and H. J. Miller

PO-47 Pectic Enzymes of Azospirillum brasiliense
C. Sekar and N. N. Prasad





POSTER SESSION B
Tuesday, May 9, 1989

**Mycorrhizae,
Microbial Plant Protectants,
Siderophores, and
Biochemistry of Plant-Microbe Interactions**

Afternoon

12:30 - 1:30	Set-Up
3:30 - 4:00	Set-Up
4:00 - 6:00	Poster Viewing and Discussion
4:30 - 5:15	Authors of odd numbered posters (PO-51, PO-53, etc.) present.

Evening

9:00 -11:00	Poster Viewing and Discussion
9:00 - 9:45	Authors of even numbered posters (PO-50, PO-52, etc.) present.

Please take down Tuesday Posters by 1:30 pm, Wednesday.

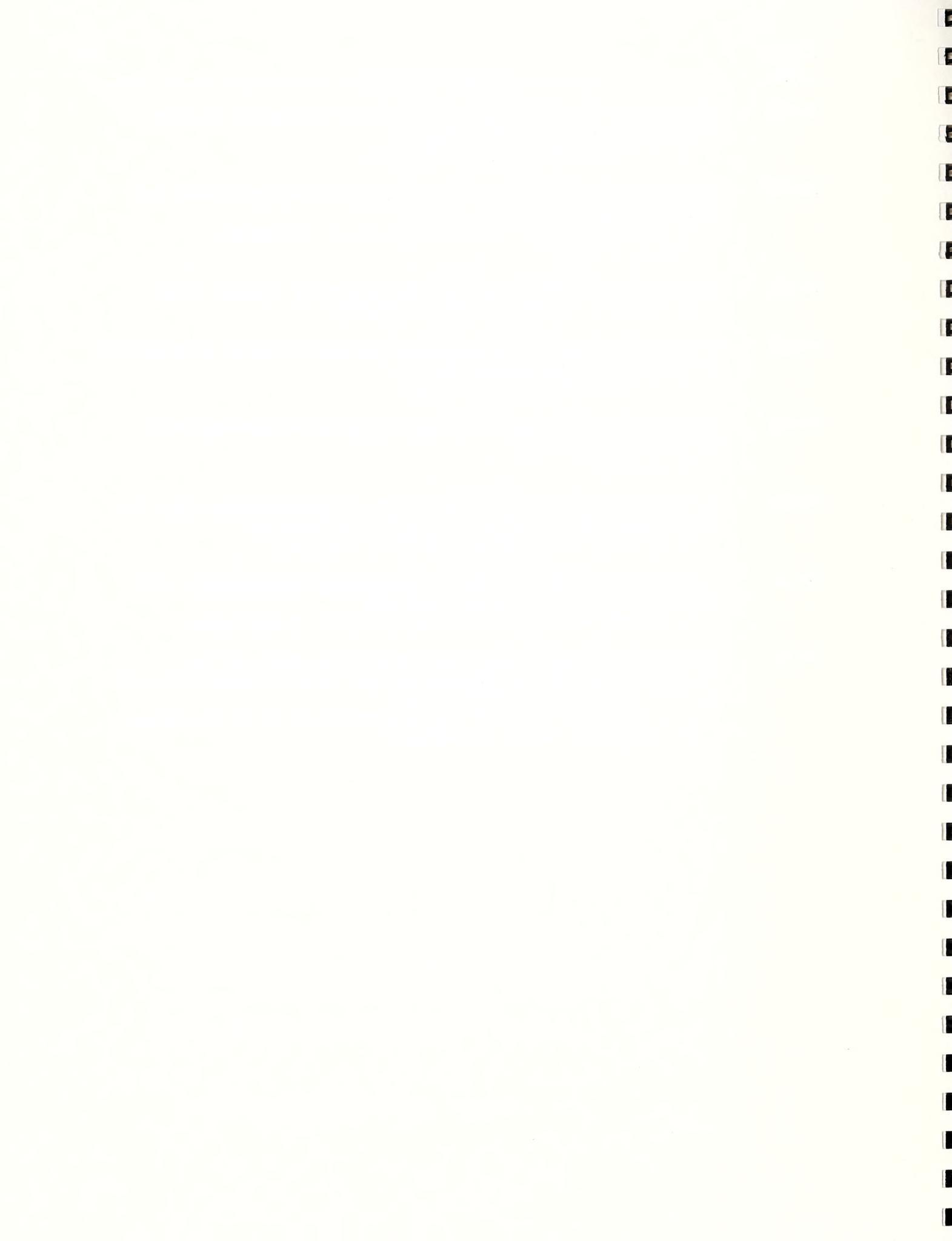
Poster No.	Title and Author
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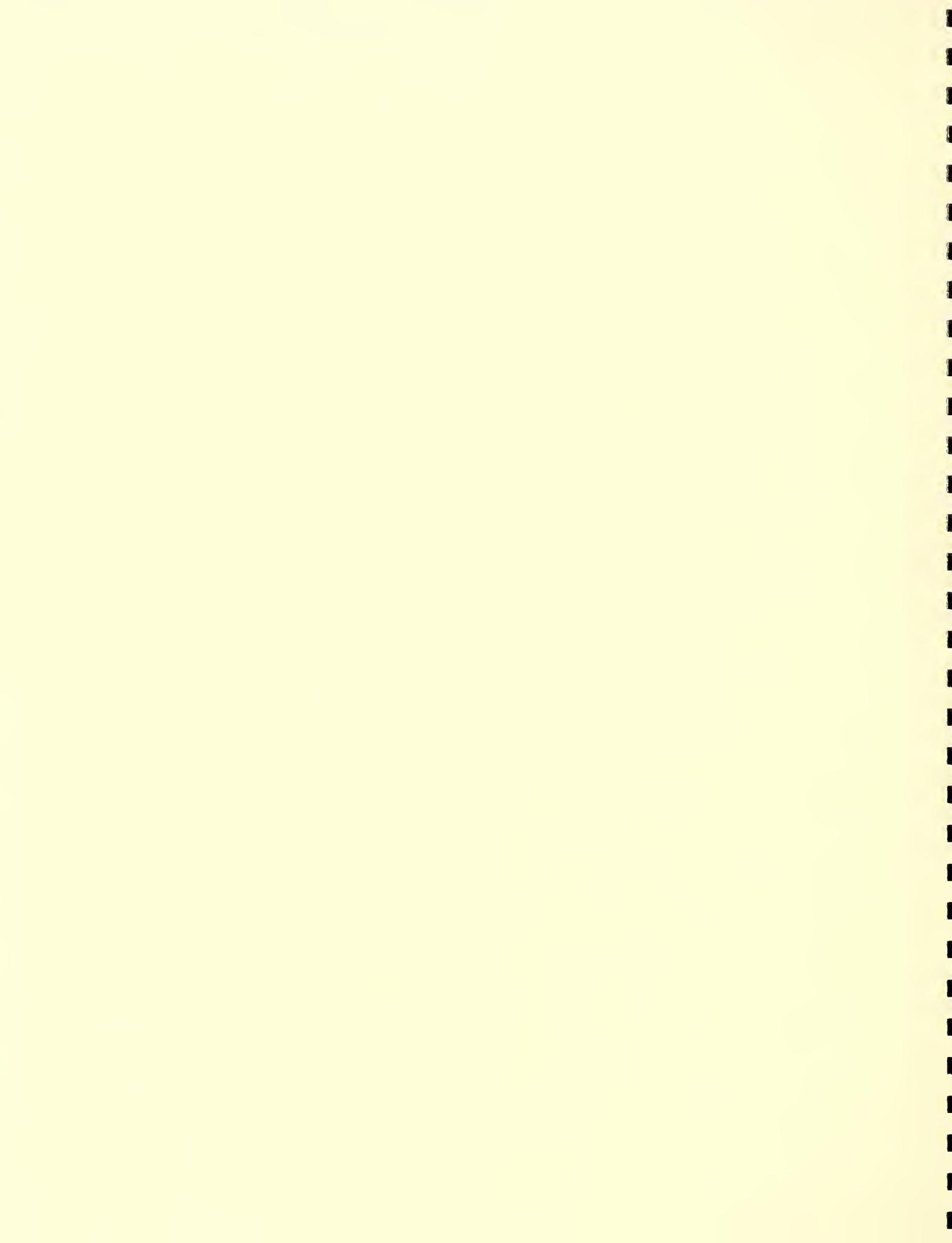
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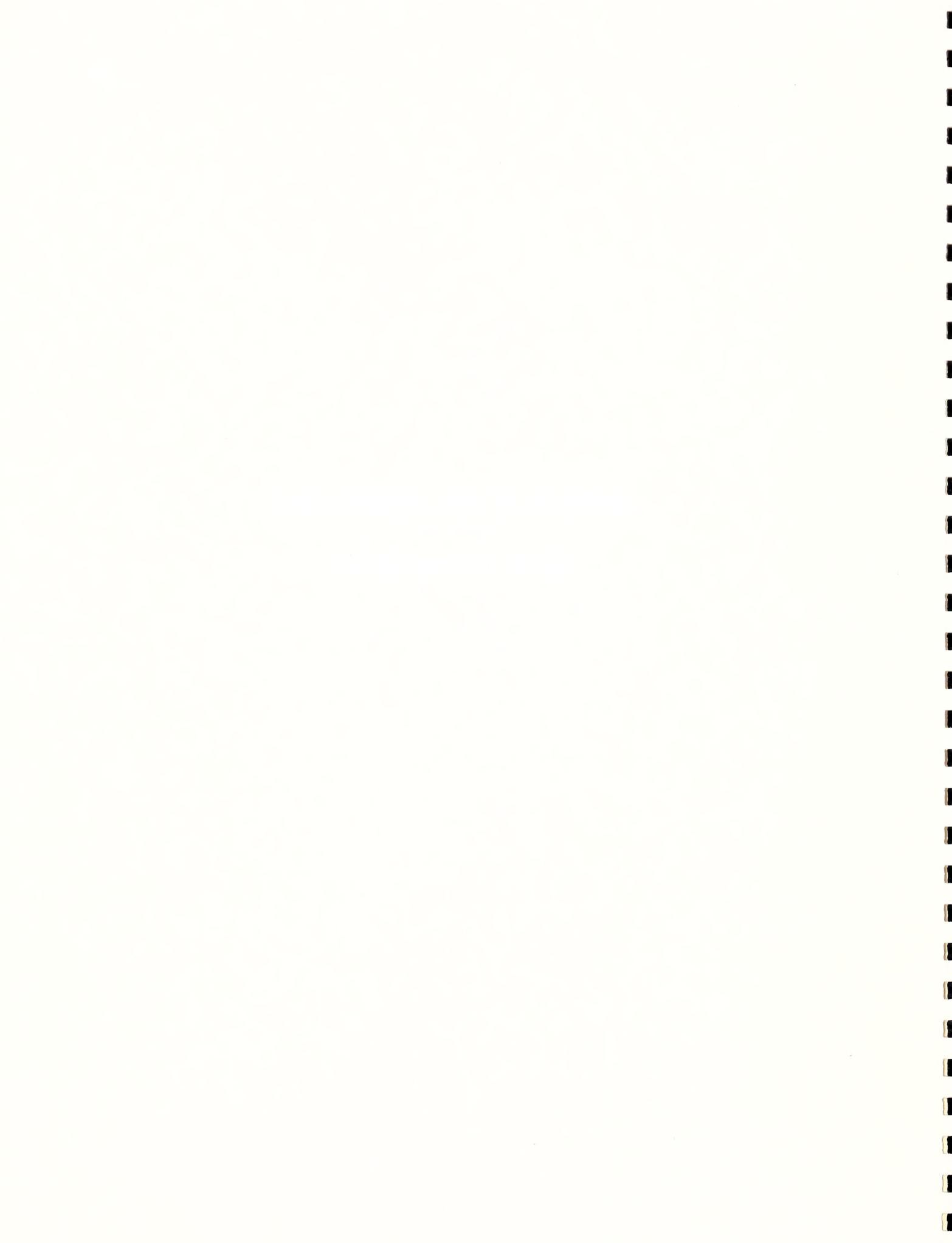






ABSTRACTS OF ORAL PRESENTATIONS

(OR-01 THROUGH OR-41)



OR-01**RHIZOSPHERE RESEARCH - 85 YEARS OF PROGRESS AND FRUSTRATION**

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A symposium on "The Rhizosphere" should commence with Lorenz Hiltner who, in 1904, reported greater numbers of bacteria around the roots of legumes than in the bulk soil. He hypothesized that this greater microbial activity near roots could affect nutrition which led to expectations that an improved understanding of the microbiology of the rhizosphere would increase crop production. Land-mark studies by Starkey, Lochhead, Katzenelson and Clark described the quantitative and qualitative composition of the rhizosphere. Our understanding of the rhizosphere has since improved through techniques such as axenic plant culture, radioisotopes, electron microscopy, selective media and antibiotic resistance markers. We have information on the release of organic materials from roots, the intimate associations between roots, soils and microbes as seen by electron microscopy, and the movement of introduced bacteria. Although root pathologists through rotations, resistant varieties, and chemicals, and rhizobiologists, through seed treatment, have modified the rhizosphere microflora and improved plant growth, attempts to modify the general rhizosphere microflora by introducing beneficial organisms have been frustrated by variable responses. These frustrations have arisen because much of the research tried to bypass the rhizosphere with scant attention to the ecology of native and introduced micro-organisms. There are exciting prospects in applying modern techniques such as DNA probes, monoclonal antibodies, and genetically engineered micro-organisms to rhizosphere studies. This will enable us to alter the microflora and improve plant performance through root disease control, plant growth stimulation, improved nodulation and nitrogen fixation, and increased nutrient uptake.

OR-02**SUBSTRATE FLOW IN THE RHIZOSPHERE**

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The major source of substrates for microbial activity in the ectorhizosphere and on the rhizoplane are rhizodeposition products. Depending on plant species, age and environmental conditions, these can account for up to 40% (or more) of the dry matter produced by plants. They are composed of exudates, lysates, mucilage and secretions, dead cell material, as well as respiratory CO_2 . The microbial populations colonizing the endorhizosphere, including mycorrhizas, pathogens and symbiotic N_2 -fixers have even greater access to the total pool of carbon derived in photosynthesis. Utilization of rhizodeposition products induces at least a transient increase in soil biomass but a sustained increase depends on the state of the native soil biomass, the flow of other metabolites, from the soil to the rhizosphere and the water relations of soil. In addition, the phenomena of oligotrophy, cryptic growth, plasmolysis, dormancy and arrested metabolism can all influence the longevity of rhizosphere organisms. With this background, microbial growth in the rhizosphere will be discussed.



OR-03**MICROBIAL DYNAMICS IN THE RHIZOSPHERE**

Glynn Bowen

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The composition of the rhizosphere microflora, and successful introduction of selected micro-organisms depends very largely on the ability of the introduced micro-organism to move along the root or with the root cap and compete with the microbial inoculum from the soil. Studies on migration/movement will be reported. Colonization will, in turn, affect the rhizosphere microbial composition in subsequent crops. A large component of poor sustained competitiveness of many introduced rhizobia with time may be due largely to their poor migration along the root and their spatial restriction in soil. This paper also examines relationships of soil populations of particular organisms to rhizosphere colonization and 'desirable' characteristics of successful inoculated micro-organisms, stressing experimental approaches. The use of single character mutants has enabled the experimental assessment of the importance of particular characteristics e.g. flagella, importance of agglutinin, production of hormones etc. Contrary to general belief, the importance of flagella and simple agglutinin properties maybe relatively minor in rhizosphere competence, but (non-infective) organisms exhibiting phase 2 adhesion (Dazzo *et al.*, 1984) may have particular importance as a commensal relation. These and other factors in rhizosphere colonization will be discussed, as will the use of 'microbial probes' in rhizosphere studies.

OR-04**PLANT ROOT GROWTH AND DEVELOPMENT**

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Much of the detailed basic research on root growth and development was undertaken during the early portion of this century. Recent research has explored some of the underlying anatomical, physiological and genetic mechanisms driving root growth. There have been three major constraints to plant rhizosphere research 1) inconsistent use of an inexact terminology, 2) inadequate information about different root types (morpho-types) and their functional relationships, and 3) extreme variability. Information is accumulating which suggests that although roots all have apparently very similar anatomies, their detailed anatomy may differ dramatically, and their functions may be very different. The concept of continual growth and die-back of small roots impacts on the understanding of the bases for soil flora and faunal biodynamics. That some roots may have no active role in nutrient or water uptake forces a re-evaluation of even the most tightly held concepts. This paper suggests that genotype by environment interaction (a necessary evolutionary and adaptive condition for plant rooting) is responsible for the observed extreme variability. This variability has forced the conclusion that rooting patterns are under multi- or poly-genic control. However, single gene root mutants discovered in tomato and statistical analyses designed to handle genotype by environment interaction suggest that this perceived genetic complexity is conditioned by a simple genetic system with extensive genotype by environment interaction.



OR-05

ROOT COLONIZATION BY INTRODUCED AND INDIGENOUS
MICROORGANISMS

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Root colonization involves two phases: dispersal of microorganisms from a source of inoculum to the actively growing root, and multiplication or growth in the rhizosphere. Soil physical, chemical, and biological factors have been shown to affect root colonization, but the phenotypic attributes of plants and microorganisms which contribute to successful root colonization generally have not been identified. Quantitative studies on the distribution of root colonists in time and space are needed to develop mathematical models to describe the root colonization process. This would enable more effective management of rhizosphere populations to achieve biological control and enhance plant growth.

OR-06

GENETIC APPROACHES FOR STUDYING RHIZOSPHERE COLONIZATION

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We are studying colonization of plant roots by bacteria, using a Pseudomonas-wheat system as the model. Most bacterial traits involved in colonization are yet to be defined. We have initiated studies to identify bacterial genes which play significant roles in this process. The general approach is to use transposons to construct collections of insertion mutants, each of which is then screened for alterations in their interactions with the host plant. We shall discuss two examples. 1) A Tn5 derivative containing a constitutively expressed beta-galactosidase (lacZ) gene was used to generate a collection of insertion mutants, all of which can now be distinguished from the wild-type parent on X-gal plates. Each mutant was examined for its ability to colonize wheat seedlings in the presence of the wild-type parent (competitive colonization assay). Mutants which gave wild-type:mutant ratio of 20:1 or greater were examined further. The competitive colonization assay is also being used to examine the relative competitiveness of different bacterial isolates and the factors involved. 2) A Tn5 derivative which carries a promoterless lacZ gene located near one end of the transposon was constructed. Expression of the lacZ gene depends on the presence of an active promoter outside of the transposon in the correct orientation. Insertion mutants generated with this transposon were examined for changes in beta-galactosidase expression in the presence and absence of plant root exudate. A number of mutants which showed differential lacZ expression have been identified and are being characterized. Bacterial genes which respond to plant root exudate may play significant roles in colonization. Characterization of such genes will contribute to the understanding of the process.



OR-07

FATE OF GENES AND GENETICALLY ENGINEERED MICROORGANISMS
IN SOIL AND RHIZOSPHERE

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Nucleic acid based hybridization methods have been developed to track specific genes and microorganisms in the soil-plant system. These methods are based on the recovery of DNA from the soil or rhizosphere. The rapid advances in molecular biology methods has resulted in continual improvements in the sensitivity, quantitation, and ease of use of these methods. Application of M-13, PCR, hexamer priming, and direct beta quantification methods to population detection in soil will be presented. We have used engineered and native Pseudomonas and Bradyrhizobium strains in model soil core systems, with and without plants, to develop methodology for in situ microbe detection. The nucleic acid based methods allow the detection of a number of organisms in the same sample, which can not usually be done by selective plating.

OR-08

USE OF MUTANTS TO STUDY THE IMPACT OF CHEMOATTRACTION TO SLOUGHED ROOT CAP CELLS ON PATHOGEN POPULATIONS IN THE RHIZOSPHERE

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Lysates from dead or dying sloughed root cap cells have long been considered to be important sources of organic matter in the rhizosphere. Recently, it has been shown that root cap cells can be induced to divide and grow in culture (Hawes and Pueppke 1986), and that they can survive for some time in the rhizosphere (Vermeer and McCully 1982). I have found evidence that root cap cells may not serve merely as passive sources of nutrients, but instead may play a previously unrecognized role in regulating microbial populations in the soil. The intact cells exhibit selective properties with respect to binding and chemotaxis that are distinct from properties of cell lysates, and the interactions vary among different host-pathogen combinations. For example, Pseudomonas solanacaerum cells bind to living but not dead cucumber root cap cells, whereas Agrobacterium tumefaciens cells bind equally to both, but do not bind at all to oat root cap cells, regardless of their condition. Zoospores of some fungal pathogens are chemotactically attracted to cell extracts but not to intact cells, while other fungal zoospores are attracted to both. In several cases, the phenotype of the whole plant with respect to susceptibility and resistance to infection is expressed in root cap cell populations. I am using mutants of A. tumefaciens to test the hypothesis that chemotactic recognition of root cap cells of pea influences pathogenesis and rhizosphere colonization.



OR-09 MICROBIAL AUTECOLOGY IN THE SOIL RHIZOSPHERE

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Most microbiological studies of the rhizosphere during the 85-year period since Hiltner coined the term "rhizosphere" have been devoted to the general characterization of the "rhizosphere effect" and the isolation of organisms from roots. The reality of biotechnology as a force to alter agriculture and society now demands a sharp focus on the biology and ecology of the soil rhizosphere. Plants modified by molecular genetic techniques for specific attributes have already reached the field test stage, as have similarly modified microorganisms. The two meet in the rhizosphere where their interactions must be studied predominantly at the autecological level. The questions relate to the specific microorganism of interest: how it enters and integrates into the rhizosphere community, survives to accomplish its special mission, and how it disseminates from the rhizosphere. Some current and potential approaches to the autecology of organisms in the rhizosphere are discussed.

OR-10 APPLICATIONS AND LIMITATIONS TO RHIZOTRONS AND MINIRHIZOTRONS

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Rhizotrons and minirhizotrons are transparent wall techniques that allow the researcher to observe the same plant roots and their rhizoplanes and rhizospheres on an intermittent basis while the roots are growing in soil. Rhizotrons are underground cellars with walls of a transparent material. Soil is located immediately behind the wall. Plant roots will grow to and sometimes along the wall-soil interface and can be observed with the unaided eye or by using a microscope. Time lapse photography can be used to study time sequences of reactions that occur at or near the interface. You can see roots shrinking and swelling as the soil water potentials around the roots decrease and then increase or you can see roots break as the soil shrinks. You can study how nematode masses collect and disperse or you can see root hairs form and then die. In some plants, light shining on the roots will decrease the amount of roots visible at the interface, when compared to nearby bulk soil. The minirhizotron technique involves placing a transparent tube into the soil profile at a small angle from the vertical then inserting a light source and some type of viewing apparatus into the tube. The most common viewing apparatus now used is a color video camera. A microscope attachment is available for the camera. The images are then recorded on a portable video recorder. When compared to the rhizotron, the minirhizotron technique greatly reduces the amount of light that falls on the roots and also can be used in naturally structured soils. Both techniques are excellent for studying dynamics of microflora and microfauna development on the rhizoplane or in the rhizosphere.



OR-11

IMAGE ANALYSES OF NMR AND VIDEO RECORDED ROOT SYSTEMS

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Nuclear magnetic resonance (NMR) imaging of water-hydrogen and the computer analysis of video recorded root images from minirhizotrons quantify root length, width, and branching larger than 150 μm . Changes in these images can be calculated to provide methods for studying the spatial and temporal development of roots. Mixtures of quartz sand:peat:kaolinite clay (5:3:2) or the selection of soil series with low ferromagnetic (LFM) contents eliminate the NMR masking problems associated with most natural soils. LFM soils also enhance the visualization of water within roots and reduce the visualization of soil water as the matric water potential approaches 0.1 MPa. Roots in video-recorded images from minirhizotrons or washed root samples can be separated from associated background materials, and interferences. Root length and branching can be evaluated by width classes using calibrated skeletonizing algorithms. Both non-invasive NMR and non-destructive minirhizotron techniques can be standardized with the more traditional destructive methods of root analyses.

OR-12

THE DEVELOPMENT OF A CONCEPTUAL MODEL OF ROOT GROWTH

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A process-oriented model is presented for the description of root growth in dicotyledonous seedlings with taproots. The model is being developed to describe the relationships between root growth and colonization of the root by various microorganisms and infection by soilborne pathogens. The model is based on processes depicting the initiation and development of root segments. A root segment is defined as that portion of a root between successive branches or from the most terminal branch to the root tip. Each segment is identified by its root order, according to the morphometric root analysis system, and root type. Three root types are recognized: taproot, primary and secondary lateral roots, and basal roots. The model begins with planting, progresses through seed germination and taproot formation, primary and secondary lateral branching, and basal root initiation and branching. The time delays between successive stages are major determinants of the dynamics of the model and are represented by Bessel delay networks. The model is adaptable to fibrous root systems and monocotyledonous seedlings.



OR-13

CONVERSION FACTORS TO USE THE THYMIDINE METHOD
WITH RHIZOSPHERE BACTERIA

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Bacterial growth rates can be calculated when subsamples of soil in different proximity to roots is labeled with ^3H thymidine and incorporation in DNA measured. The constant relating no. of cells to mole of thymidine, is to be direct measured or empirically derived. This was done with exponential growing soil and rhizosphere bacteria and the labeled macromolecules separated. Pseudomonas chlororaphis and isolates of P. putida incorporated in total very little ^3H thymidine, less than 40% of labeled macromolecules found as DNA, the major labeled fraction was RNA. Growth at 10C or addition of adenosine did not change this pattern. Strains of Escherichia coli other isolates than pseudomonads, and mixed populations, incorporated nearly all ^3H thymidine into DNA at high levels with empirically conversion factors $0.3-3.0 \times 10^{18}$ cells/mole thymidine comparable to direct DNA measurements by flow cytometry, $0.07-1.0 \times 10^{18}$ cells/mole thymidine.

OR-14

Legume Inoculation, Successes and Failures

R. H. Miller and S. May, N. Carolina State University

The contribution of leguminous plants including food, forage and tree legumes to the nitrogen needs of agricultural systems is well recognized. At the same time the efficacy of inoculation with specific rhizobia to improve biological nitrogen fixation remains less certain. In a historical perspective it is interesting to note that attempts at improving N-fixation through inoculation of soil or seed began almost simultaneously with the successful demonstration that symbiotic N-fixation was associated with legume root nodules. At the same time the problems which plagued these early investigators, i.e., differences in response of method, of inoculation, problems in assuring adequate inoculant quality, excessive claims of efficiency by some inoculation producers and the inability of inoculant rhizobia to compete against indigenous rhizobia, remain with us today. This is true even though our journals contain a voluminous literature covering inoculation trials, the intricacies of the symbiosis, methods of inoculation, rhizobium ecology, the genetics of rhizobia, etc. The challenge to successfully and selectively introduce selected or genetically engineered highly effective rhizobium strains remains a high priority.

OR-15

IMPORTANCE OF SAPROPHYTIC COMPETENCE FOR
INTRODUCED MICROORGANISMS

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In legumes of agricultural importance nitrogen fixation is carried out predominantly by members of poorly characterized soil populations of Rhizobium and Bradyrhizobium. As a result of inconsistency in the performance of inoculant rhizobia, members within soil populations are hypothesized to possess undefined phenotypes which can confer superior competitiveness at nodulation, and/or superior saprophytic capabilities in soil and rhizosphere habitats. At this time, however, there is no direct evidence to support a logical hypothesis that possession, absence, or variable expression of a particular phenotype correlates with success or failure either as a soil saprophyte or during the symbiosis-establishing time period. Data will be presented from field and laboratory soil studies which describe our current understanding of the structure of Rhizobium soil populations. Information will be presented which describe the influence of nutritional and abiological factors upon the viability and physiological activity within the different serogroup populations of a soil Rhizobium population.

OR-16

ENHANCING THE COLONIZATION OF SELECTED BACTERIA IN
THE RHIZOSPHERE: THE RHIZOBIUM EXPERIENCE

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Studies have been conducted to determine why rhizobia fail to become major colonists in the legume rhizosphere and to devise means to enhance their colonizing ability. Several abiotic stresses appear to be important, and means have been devised to overcome some of these constraints. Competition with indigenous rhizosphere bacteria is a major factor minimizing the extent of colonization by rhizobia, and protozoan grazing also may deleteriously affect their numbers. The severity of these biotic stresses may be markedly reduced by use of pesticides that suppress indigenous bacteria or protozoan grazers and inoculation with rhizobia that are made resistant to the inhibitory compounds. Systemic fungicides are particularly attractive for this purpose. Colonization by introduced bacteria may also be promoted by co-inoculation of the rhizobia with bacteria that produce antibiotics that influence indigenous rhizosphere organisms but to which rhizobia are made resistant.



OR-17

FLAVONOID NODULATION SIGNALS RELEASED BY ALFALFA

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Some flavonoids released from alfalfa seeds and roots induce transcription of nodulation (nod) genes in Rhizobium meliloti. Luteolin, a compound identified in alfalfa seed extracts, and some commercially available flavonoids are known nod inducers. An understanding of the rhizosphere, however, requires that flavonoids actually present in that environment be identified. We report here results of spectroscopic analyses (UV-visible, ¹H-NMR, and MS) that identify nod-inducing flavonoids actually released from alfalfa seeds and roots. One compound is active at a much lower concentration than luteolin in bioassays of a nodC-lacZ fusion in R. meliloti. Different flavonoids are released from seeds and roots. Understanding how these alfalfa flavonoids interact with various nodD alleles and their effect on rhizobial chemotaxis will help define biological events occurring in the seed zone and rhizosphere.

OR-18

GENETIC STABILITY IN BRADYRHIZOBIUM JAPONICUM IN THE FIELD

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Genetic instability within strains of rhizobia maintained on laboratory media is well recognized, although only rarely has the mutation been characterized. Variability within a strain, following its introduction to the field, is very difficult to recognize due primarily to our poor understanding of any naturally-occurring populations of rhizobia. In Australia, the soils are naturally deficient in Bradyrhizobium japonicum and since 1966, only one strain (CB1809 = USDA136b) has been used in commercial inoculants; prior to that, soybean cultivation (and inoculation) was limited to very small areas. This situation provides an excellent opportunity to examine stability/variability within field populations of strain CB1809. Field isolates from different soils have been made following plant entrapment or directly from the soil. Symbiotic tests have indicated little or no change in nitrogen-fixing effectiveness or in the hup (hydrogen uptake) characteristic. Restriction fragment length polymorphism analyses, using the λ -probe (Hahn and Hennecke, Appl.Env.Microbiol. 23,2253,1987) on 50 isolates, have failed to detect differences, even after eight years in the field. However, marked changes in serological characters have been observed. These results will be compared with U.S. data for B.japonicum.

OR-19

CHEMOTAXIS OF RHIZOBIA TO ROOT PHENOLICS THAT INDUCE SYMBIOTIC GENE EXPRESSION

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Rhizobia are soil bacteria which symbiotically infect legume roots and generate nodules in which they fix atmospheric nitrogen for the plant in exchange for photosynthetically fixed carbon. A crucial aspect of signal exchange between these symbionts is the secretion of phenolic compounds by the host root which induce nodulation gene expression in the bacteria. Stimulation of nod gene expression by host phenolics is required for nodule formation, is biochemically specific at 10^{-6} M concentrations, and is mediated by nodD. We and others have shown that rhizobia display chemotaxis to 10^{-9} M concentrations of the same phenolic compounds. Chemotaxis to inducer phenolics is selectively reduced or abolished by mutations in certain nod genes governing nodulation efficiency or host specificity. Conversely, mutations in rhizobia that affect general motility or chemotaxis have substantial effects on nodulation efficiency and competitiveness. These findings suggest that microbes entering the rhizosphere environment may utilize minor, non-nutrient components in root exudates as signals to guide their movement towards the root surface and elicit changes in gene expression appropriate to this environment.

OR-20

GENE-FOR-GENE INTERACTION IN THE LEGUME-RHIZOBIUM SYMBIOSIS

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As originally defined, the gene-for-gene hypothesis describing certain plant host-pathogen interactions proposed that for "each resistance gene in the host there is a specific, complementary gene conditioning pathogenicity (virulence) in the parasite." The original proposal, as well as most subsequent reports have indicated that a resistant phenotype is obtained only when a dominant gene for resistance in the host interacts with the complementary dominant gene for avirulence in the pathogen. Thus, the gene for virulence in the pathogen is recessive. The rationale for this being that virulence results when the pathogen no longer produces some elicitor compound which triggers the defense system of the host. While this genetic model is adequate to describe parasitic relationships, it may not be applicable to symbiotic interactions. Our goal is to facilitate the positive interaction of specific paired soybean-Bradyrhizobium combinations by exploiting host and rhizobial genetic factors. Therefore, understanding the nature of the gene-for-gene interaction in this symbiosis is of critical importance to the attainment of our research goal.

OR-21

MICROBIAL INFLUENCE ON GENE-FOR-GENE INTERACTIONS IN
LEGUME-RHIZOBIUM SYMBIOSSES

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Recent advances in our understanding of the molecular genetics of legume-Rhizobium symbioses have indicated that relatively few bacterial genes are required for nodulation. While some of these genes are functionally similar and shared among microsymbionts nodulating genetically diverse legumes, others appear to encode host-specific nodulation (hsn) functions which allow for nodulation of plants in a given legume genus. More recently, genotype-specific nodulation (GSN) determinants have been identified in R. leguminosarum bv. viceae strain TOM and in B. japonicum strain USDA 110. GSN determinants refer to those bacterial sequences which allow for nodulation of specific plant genotypes within a given legume species. In contrast to the avr loci of several plant pathogens, rhizobial host-range determinants (hsn and GSN) have been shown to positively affect nodulation. That is, the introduction of exogenous hsn and GSN loci extends host-range. Since GSN loci have been reported to interact with single host plant alleles, it suggests that gene-for-gene interactions occur in rhizobial-legume symbioses and contribute to nodulation specificity at the host genotype level.

OR-22

TRIPARTITE INTERACTIONS BETWEEN LEGUMES, RHIZOBIA AND
MYCORRHIZAL FUNGI.

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Colonization of plant roots by vesicular-arbuscular mycorrhizal (VAM) fungi has fundamental, and as yet not-well understood effects on the physiology and morphology of the host plant and on the functions of root nodules. When legumes are grown in soils limiting in P, the VAM effect may mimic that of P fertilization and is therefore often mistaken for a P effect. While P nutrition may be dramatically improved by the VAM condition, the uptake of other nutrients is also affected and may range from enhancement to inhibition mediated either directly by the symbiotic association or by secondary changes produced in the rhizosphere. The mechanism of control over changes in the development of VAM plants, such as the root-shoot ratio, and specific root length, leaf area and nodule mass is little-known and may be influenced nutritionally or through the production of phytohormones. Phenomena of interest in crop productivity are the VAM-mediated changes in leaf gas exchange including water-use efficiency, in nodule gas exchange, in the P-use efficiency of CO₂ and N₂ fixation, and in plant water status. In soil conservation, the export of carbon compounds to soil microsites not reached by the roots appears to enhance soil aggregation.

OR-23

EXUDATE MOLECULES INITIATING FUNGAL RESPONSES TO SEEDS AND ROOTS

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Plant pathogenic fungi survive in soils in a quiescent state. In order for root-pathogen interactions to be initiated, dormant propagules must be activated by molecules present in seed and root exudates. Without the release of such stimulatory molecules, the majority of root infections do not occur. Currently, little is known about the specific molecules involved in stimulating propagule germination and initiating root-pathogen interactions. Although certain molecules can be shown to elicit germination responses in vitro, responses of propagules reared on conventional culture media do not always reflect the responses of those formed on plant tissues in soil. The interaction of Pythium species with germinating seeds has served as a model to answer questions about propagule behavior and the role exudate stimulant molecules play in establishing root-fungus interactions. The identity and function of volatile and water-soluble molecules in propagule germination are discussed.

OR-24

EFFECTS OF DELETERIOUS AND HCN-PRODUCING PSEUDOMONADS ON RHIZOSPHERE INTERACTIONS

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Rhizobacteria live around roots, but also inside the cortical root tissues, by utilizing organic substances released from root cells into the intercellular spaces and the root environment. Effects of metabolites of these rhizosphere inhabiting bacteria on root physiology and plant development have hardly been studied. Recent studies, however, indicate that, depending on environmental factors and plant species, certain strains of rhizosphere Pseudomonas spp. and some of their metabolites, such as HCN, may inhibit or enhance plant establishment and development of plant disease. Cultural practices, such as cropping frequency, no tillage, and soilless cultivation, as well as edaphic factors seem to determine these rhizosphere interactions.

OR-25

FORMULATION AND DELIVERY SYSTEMS OF BIOCONTROL AGENTS WITH EMPHASIS ON FUNGI

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Soilborne pathogens infect plants in the spermosphere or rhizosphere. Effective biocontrol must occur before infection. For commercial development of microbials, obstacles in growth, formulation, and delivery must be overcome. Most of the research described is with the biocontrol fungi Trichoderma and Gliocladium and with the potential antagonists Laetisaria, Stilbella, and Cladorrhinum. Fermentation media for growth should contain easily available, inexpensive agricultural products. Biomass of Trichoderma and Gliocladium, abundant in chlamydospores, is obtained after growth on molasses, brewer's yeast, and/or various seed meals. Dry, powdered biomass is mixed with an inert carrier prior to application to soil or tuber, or the biomass is blended with alginate or carrageenan and dripped into a gellant to form beads which dry as pellets. Pellets are also prepared with the biocontrol bacterium Pseudomonas. A system is also described in which biomass is mixed with vermiculite or celatom, dried, stored, and "activated" before addition to soil for prevention of diseases caused by Rhizoctonia solani and Sclerotium rolfsii. Formulations also made with bran, peanut hull, and soy fiber containing actively-growing hyphae of fungi prevent diseases caused by R. solani and S. rolfsii.

OR-26

FORMULATION, DELIVERY SYSTEMS, AND MARKETING OF BIOCONTROL AGENTS AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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From formulation through field evaluation and marketing, biological disease control agents and PGPR's provide a special difficulty; they are alive. This fact dictates special consideration for both strain selection and how it will be prepared and packaged for the real world of agriculture. These inoculants must be produced, stored, delivered, and ultimately used by the grower. Therefore, the inoculants must be maintained at proper concentrations on a carrier over significant time periods. Upon delivery the carrier must also enable the inoculant to reproduce itself and colonize the target plant. Upon application, protection from environmental factors may also be required. Additionally, the product must be in a formulation that is compatible with the grower and his practices. These considerations must all be taken into account in order to develop a product that is not only efficacious and cost effective against chemicals with which it may compete, but also a product that will be used by the consumer. Approaches to successfully develop and deliver biopesticides and PGPR's to the agricultural market will be discussed.



OR-27

INDUCTION OF RHIZOSPHERE COMPETENCE IN THE BIOCONTROL FUNGUS
TRICHODERMA

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Apparently wild types of Trichoderma spp. do not possess the attribute of rhizosphere competence (RC); i.e. they cannot colonize the rhizosphere adjacent to developing roots from a seed treatment. To enhance RC, strains of T. harzianum were exposed to N-methyl-N-nitro-N-nitrozoguanidine and mutants selected for benomyl resistance. When benomyl was added to soil, these mutants applied to seeds colonized the rhizosphere 8 cm below the seed at densities of 10^6 cfu/g rhizosphere soil. Unexpectedly, RC was induced when benomyl was not added to the soil. There is no evidence that benomyl resistance is related to RC; however, a perfect correlation was observed between increased cellulase production by mutants and RC. This suggested that mutants utilized the mucigel as a substrate. Mutation to RC and faster growth rate, comparable to root elongation, was induced in a slow growing species like T. polysporum. Mutants with RC also were more efficient biocontrol agents and induced greater increased growth responses from a seed treatment than wild-type parents.

OR-28

MECHANISMS OF BIOCONTROL OF SOILBORNE PLANT PATHOGENS
WITH RHIZOBACTERIA

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Serratia marcescens was found to be an efficient biocontrol agent of Sclerotium rolfsii and Rhizoctonia solani under greenhouse conditions. Populations of 10^5 or 10^6 CFU g⁻¹ soil were the most effective in disease control. The highest population density of the bacteria in the rhizosphere was found on the proximal portion of the root, decreasing significantly until the tips, where they increased again. The isolated serratia was found to possess chitinolytic activity and was able to release N-acetyl D-glucosamine from cell walls of S. rolfsii. The gene coding for chitinase was cloned into Escherichia coli and the enzyme was uniquely excreted from the bacterium into its growth medium. When S. rolfsii was sprayed by partially purified chitinase produced by the cloned gene, rapid and extensive bursting of the hyphal tips was observed. This chitinase preparation was found to be effective in reduction of disease incidence caused by S. rolfsii in beans and R. solani in cotton, under greenhouse conditions. A similar effect was obtained when a viable E. coli cell containing the plasmid with the chitinase gene (pLCHIA), was applied.



OR-29

MECHANISMS OF BIOCONTROL OF SOILBORNE PLANT PATHOGENS WITH FUNGI

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Determining mechanisms by which antagonistic fungi control plant pathogens is an important step in developing biorational approaches to disease management. Knowledge of mechanisms can be used to design screening procedures for potential antagonists, evaluate management practices which favor antagonists, select genetically improved antagonists, and assist in EPA registration of antagonists. These approaches should increase the probability of successful, consistent biocontrol in the field. Mechanisms of biocontrol include competition, parasitism, and antibiosis, all of which function in the rhizosphere. Competition may be the mechanism most prevalent in natural systems, but it is often difficult to document.

Parasitism can be an efficient control mechanism and has the potential to be very target specific, as is the case with control of Sclerotinia species by Sporidesmium. Antibiosis has not been definitively demonstrated to be the sole control mechanism of any biocontrol fungus, although there is evidence for involvement of antibiotics, antibiotic-like compounds or enzymes in control by numerous fungi including Trichoderma, Gliocladium, and Talaromyces. These mechanisms are not mutually exclusive; different mechanisms may act in concert to produce biocontrol.

OR-30

IDENTIFICATION AND REGULATION OF GENES INVOLVED IN THE BIOCONTROL OF SOIL-BORNE PLANT PATHOGENS WITH FLUORESCENT PSEUDOMONADS.

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The identification and elucidation of the functional role of both regulatory and structural genes central to biocontrol efficacy will be essential for optimizing the performance of microbial pesticides. Relatively few different systems have been employed to permit the *in vitro* and *in situ* analysis of the regulation of genes responsible for the biosynthesis of antifungal compounds. These "reporter gene" systems (β -galactosidase and bioluminescence) allow the indirect quantitative measurement of native gene induction and transcriptional events as well as the effects of the introduction of a constitutive promoter on gene expression. The influence of various environmental parameters on expression and efficacy of Oomycin A, an antifungal compound from P. fluorescens strain Hv37a, have been investigated using these approaches. The experience gained with Hv37a has provided insights for developing strategies for their application to other beneficial strains.



OR-31

STRUCTURE, PROPERTIES AND GENETIC REGULATION OF SIDEROPHORES

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Siderophores are generally thought to be designed specifically to complex Fe(III) at high dilution and thus make the essential metal ion more available to the microbial cell. It is then somewhat surprising that the list of mono-catecholate siderophores, which would be expected to display only a modest chelate effect, continues to grow. Specific examples are amonabactin from Aeromonas hydrophila, chrysobactin from Erwinia chrysanthemi, and anguibactin from Vibrio anguillarum. However, recent work suggests that such simple complexes may be taken up on receptor mediated pathways. In Escherichia coli some progress has been made in understanding the mode of regulation, by iron, of synthesis and transport of aerobactin, a siderophore that is part of the virulence armamentarium of clinical isolates of the bacterium. The genetic determinants, whether coded on pColV-K30 or the chromosome, are organized into a single transcriptional unit. A trans-acting protein, Fur (ferric uptake regulation), complexes one or more ions of Fe(II) and then binds to an operator containing the palindromic consensus sequence 5'-GATAATGATAATCATTATC to negatively block transcription. The same system controls expression of several additional genes in E. coli, and the fur mutation imparts a number of phenotypes.

OR-32

ROLE OF ANTIBIOTICS AND SIDEROPHORES IN BACTERIA

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Both antibiotics and siderophores have been implicated in the control of soilborne plant pathogens by fluorescent pseudomonads. In Pseudomonas fluorescens 2-79, which suppresses take-all disease of wheat, the importance of the antibiotic phenazine-1-carboxylate was established genetically, with mutants deficient or complemented for antibiotic production, and by isolation of the antibiotic from the rhizosphere of wheat colonized by the bacteria. Because this antibiotic does not fully account for the suppressiveness of strain 2-79, additional mutants were analyzed to evaluate the role of the fluorescent siderophore and of a nonphenazine antibiotic (Aff) produced when iron is limiting. Whereas strains producing only the siderophore conferred little or no protection against take-all, Aff⁺ strains were suppressive, albeit much less so than phenazine-producing strains. This study and other recent reports suggest that iron-regulated antibiotics may occur more frequently than previously recognized, and that such substances may be responsible for some of the beneficial effects previously attributed to fluorescent siderophores.

OR-33 FACTORS INFLUENCING SIDEROPHORE-MEDIATED BIOCONTROL ACTIVITY OF RHIZOSPHERE PSEUDOMONAS spp.

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Variable efficacy of biocontrol strategies among agricultural fields poses a significant obstacle to agronomic application. Any factor influencing the activity or establishment of a biocontrol agent will influence field performance. The sensitivity of the indigenous pathogen population to biocontrol may be one factor varying among agricultural fields. Certain Pseudomonas spp. strains control potato seed piece decay and soft rot diseases caused by Erwinia carotovora, due to the production of fluorescent siderophore(s). Sensitivity to biocontrol by Pseudomonas spp. varies among the target E. carotovora strains evaluated (Xu and Gross, 1986. *Phytopathology* 76:414). Studies were initiated to identify the siderophores produced by E. carotovora and their potential roles in interactions with Pseudomonas spp. All of 23 E. carotovora strains tested produced a catechol siderophore but only strain W3C105 produced the hydroxamate siderophore, aerobactin. Genes involved in aerobactin and catechol biosynthesis were identified from a genomic library of strain W3C105. We postulate that iron-acquisition systems of target E. carotovora strains may influence their sensitivities to siderophore-mediated biocontrol by Pseudomonas spp.

OR-35 RHIZOSPHERE INTERACTIONS AND SIDEROPHORES

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Certain plant growth-promoting pseudomonads inhibit deleterious and pathogenic rhizosphere bacteria and fungi by producing siderophores. Properties of a siderophore transport system which might provide a competitive advantage under iron stress conditions include ability to utilize other organisms' siderophores, higher Fe(III) stability constant, faster kinetics of dissolution of Fe(III) minerals and oxyhydroxide polymers, more efficient transport system, and resistance to degradation. In order to determine the concentration and localization of siderophores in the rhizosphere monoclonal antibodies to ferric pseudobactin, the siderophore of Pseudomonas putida B10, have been developed. One Mab did not cross react with 2 other pseudobactins. Antibiosis between P. putida B10 and Gaeumannomyces graminis var. tritici, the take-all pathogen, has been studied on a defined medium. The carbon source used is crucial to antibiosis and siderophore production, while pH and temperature are less important.



OR-36

GENETICS OF IRON TRANSPORT OF PLANT GROWTH-PROMOTING PSEUDOMONAS PUTIDA WCS358

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High affinity iron(III) transport in potato-growth promoting Pseudomonas putida WCS358 is initiated by an 86-KDa outer membrane receptor protein which appears to be specific for ferric pseudobactin 358, the native siderophore of strain WCS358. The DNA sequence of this receptor gene has been determined; the mature protein consists of 772 amino acids (86.01-KDa) with a signal sequence of 47 amino acids. The receptor protein shares strong homology with four regions of TonB-dependent receptor proteins of Escherichia coli, which suggests the presence of a TonB-like protein in strain WCS358 required for iron(III) transport. Strain WCS358 also possesses a low-affinity uptake system for ferric pseudobactin 358 and ferric pseudobactins from many other fluorescent pseudomonads. A gene encoding a putative outer membrane receptor protein for this broad specificity uptake system has been cloned. Genes coding for the biosynthesis of pseudobactin 358 and the high-affinity receptor protein for ferric pseudobactin 358 are regulated transcriptionally by iron(III).

OR-37

PLANT GROWTH PROMOTION MEDIATED BY BACTERIAL RHIZOSPHERE COLONIZERS

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Plant growth-promoting rhizobacteria (PGPR) represent a diverse subgroup of rhizosphere-colonizing bacteria. PGPR were first described for root crops in the 1970s when the use of antibiotic resistance made possible the monitoring of introduced bacteria in soil. In recent years, the host list of PGPR has grown to include barley, bean, canola (rapeseed), cotton, maize, peanut, vegetables, wheat, and woody species. In addition to increasing crop yields, different strains of PGPR can exert various effects on plants including biological control of soil-borne pathogens, promotion of legume nodulation by nitrogen-fixing rhizobia, and enhancement of seedling emergence rates. Reported mechanisms of action for PGPR have focussed on the indirect mechanisms of siderophore, antibiotic, or hydrogen cyanide production. Such indirect mechanisms reduce the population densities of deleterious microorganisms and thereby result in increased plant growth. Direct growth promotion by PGPR in the absence of deleterious microorganisms has been recently described.

OR-38 MICROBIAL PRODUCTION OF PLANT HORMONES

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Microbial production of plant hormones and precursor-inoculum interactions were studied. Indole-3-acetic acid was detected in soils incubated with L-tryptophan (L-TRP). Inoculation with Pisolithus tinctorius significantly stimulated the growth of Douglas fir when supplied with low concentrations of L-TRP to soil. Among three Azotobacter spp. and two Pseudomonas spp., the most prolific producer of cytokinins was A. chroococcum and among the precursors tested, adenine (ADE) and isopentyl alcohol (IA) were the most effective. The combination of ADE, IA plus the inoculum A. chroococcum enhanced the growth of radish and maize to a much greater degree than their application alone. Corn rhizosphere was found to be quite rich with microflora capable of producing ethylene from L-methionine (L-MET). Amino acids, carbohydrates and organic acids typically found in root exudates, were stimulatory to ethylene biosynthesis in soil. Etiolated pea seedlings exhibited the classical 'triple' response when L-MET and Acremonium falciforme were applied in combination to sterile soil or when L-MET was added to nonsterile soil.

OR-39 VA MYCORRHIZAE: THEIR PRESENCE, ROLE, AND CHARACTERIZATION

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Vesicular-arbuscular mycorrhizal (VAM) fungi are the most common fungal endophytic symbionts associated with plant roots. They form associations with a wide range of hosts in diverse soil types and climatic regions of the world. Their occurrence in agricultural production systems is described along with the effects that cultural practices have on them. The role of VAM fungi in improving the growth of plants by increasing the absorption of phosphorus and micronutrients and enhancing tolerance to drought is reviewed. The potential for commercial use of VAM is described along with the most recent advances in inoculum production technology. New approaches to identification of VAM fungi, including ELISA with polyclonal and monoclonal antibodies, isozyme analysis, and DNA fingerprinting, will be described and compared.

OR-41

FIELD MANAGEMENT OF VA MYCORRHIZAL FUNGI

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The potential for management of VA mycorrhizal fungi at a particular site depends on the factors that limit plant growth and the extent and timing of mycorrhiza formation. We are attempting to develop a procedure for predicting those sites where the benefits from the mycorrhizas are less than optimal. This involves gaining an understanding of the relationships between plant growth and soil and fungus parameters, predicting mycorrhiza formation using a bioassay, and subsequently quantifying the benefits that could result from management of the fungi. With existing knowledge it is not possible to (i) identify sites used for broadscale agriculture where the symbiosis is operating suboptimally, (ii) quantify the benefits that would result from increasing the rate and extent of colonization of roots by effective fungi, and (iii) identify the most cost-effective method for managing the fungi either by choice of agricultural practice or by inoculation with selected fungi. Our attempt to overcome these limitations has led to the identification of specific areas where further research is necessary.





ABSTRACTS OF POSTER PRESENTATIONS

(P0-01 *through* P0-92)

P0-01

Extracellular polysaccharide-deficient mutants of Rhizobium strain CIAT899 induce chlorosis in beans

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Tn5 mutants of the broad host range Rhizobium strain CIAT899 were screened on solid media for the apparent lack of extracellular polysaccharides (EPS). Ten independent EPS-deficient mutants were tested on plants and all retained the ability to nodulate beans (Phaseolus vulgaris L.) but induced interventional chlorosis on the trifoliolate leaves of the host. The chlorotic symptoms resembled those induced in soybeans by the rhizobitoxine-producing strains of Bradyrhizobium japonicum described by others. Induction of chlorosis appeared to require the presence of the mutants in the nodules of the host plant. The results suggest that there is a relationship between EPS deficiency and chlorosis induction, since all of the EPS-deficient mutants tested also induced chlorosis. The basis for the relationship between these phenotypes and the mechanism of chlorosis induction are being explored.

P0-02

RHIZOSPHERE INTERACTIONS BETWEEN RHIZOBIA AND NONLEGUMES

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Members of the Parasponia genus, in the Elm family, are the only nonlegumes known to form root nodules with Rhizobium bacteria. Although infection and nodule morphogenesis differ from that of legumes, only minor genetic changes are needed in rhizobia to initiate Parasponia nodulation. Transfer of the nodD1 gene from Rhizobium strain NGR234, which nodulates Parasponia, to the clover-specific R. leguminosarum bv trifolii strain ANU843 extends the host range of the recipient to include Parasponia. Studies using nod::lac fusions show that nod genes are induced by root extracts from Parasponia, wheat, rice, maize, cotton and sunflower in the presence of the nodD1 gene from strain NGR234. The induction of nodulation genes by root extracts from nonlegumes, other than Parasponia, did not lead to the formation of nodules. However, a strain ANU843 derivative carrying nod genes on a multicopy vector induced root hair curling on maize and rice plants 14 days after inoculation. This is an important observation because root hair curling is the first visible plant response in the infection of many legumes by rhizobia.

P0-03 PHOTOSYNTHETIC N₂-FIXING RHIZOBIUM

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Rhizobium strains BTAil and BTsrIII are capable of forming N₂-fixing stem nodules on plants of Aeschynomene and Sesbania, respectively. Physiological studies were performed with both bacteria *in vitro*. BTAil, which was isolated from Virginia sand, forms pink pigmented cultures, associated with the production of carotenoids, while BTsrIII produces white colonies. Synthesis of bacteriochlorophyll, with characteristic peaks, was demonstrated for whole and extracted cells of BTAil. When illuminated, BTAil retained viability during stationary phase although no growth was observed in C-free media. Light increased ¹⁴CO₂ uptake rate during log and stationary phases in cultures of BTAil but had no effect on BTsrIII cultures. Oxygen uptake rates were significantly decreased by the light. These results show that although both strains form stem nodules, BTAil is unique in its ability to support both N₂ fixation and photosynthesis. Based on the above results, we have tentatively named BTAil as Photorhizobium thompsonum.

P0-04 PHYSIOLOGICAL COMPARISONS BETWEEN ROOT AND STEM NODULES OF
AESCHYNOMENE SCABRA AND SESBANIA ROSTRATA

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In some legume species such as plants of genera Aeschynomene and Sesbania, Rhizobium strains can nodulate both stems and roots. As stem nodules have chloroplasts in the cortical cells, it is possible that the energy supply for nodule activity is different between stem and root nodules. Under greenhouse conditions, stems or roots of A. scabra were inoculated with strain BTAil, and S. rostrata with strain BTsrIII. Five weeks after inoculation nitrogenase specific activity, as well as GS and GOGAT, were higher in the stems than in the roots of both species. However, all activities were much higher in stem nodules of Aeschynomene, that accumulated 24% more N than root nodules. The ¹⁴CO₂ fixation activity *in vivo* was the same for root and stem nodules when the assay was performed in the dark. However, in the light it was 11 times higher in stem nodules of Sesbania and 38 times higher in stem nodules of Aeschynomene. Data confirmed that photosynthesis in cortical cells can supply energy to the nodules. However, with stem nodules, these results support our previous observation that strain BTAil is a photosynthetic Rhizobium.



P0-05 ISOLATION AND CHARACTERIZATION OF COWPEA
(VIGNA UNGUICULATA) LECTIN

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Successful initiation of the symbiotic relationship between legume plants and rhizobia depends on specific interactions between the partners. Early recognition in the rhizosphere is thought to be an important step in the initiation of such symbioses. One current theory, the lectin binding hypothesis, holds that plant lectin binds complementary rhizobial extracellular polysaccharide (EPS) to accomplish the recognition. It is known that the cowpea can be nodulated by various slow-growing rhizobia, but the cowpea lectin is poorly understood. We report on procedures to extract, isolate, and characterize a lectin from cowpea seeds. Separation by gel electrophoresis and staining with periodic acid-Schiff (PAS) reagent indicated that the lectin is a glycoprotein. The lectin agglutinated rabbit erythrocytes (RBC) processed by trypsinization and glutaraldehyde fixation, but not dog or human type O RBCs. Hemagglutination inhibition activity (HIA) data showed that the lectin belongs to the galactose binding family. HIA of various rhizobial EPS preparations are reported. Differences between the cowpea lectin and the galactose-binding soybean agglutinin are presented. The possible existence of isolectins in the cowpea is addressed.

P0-06 DISTRIBUTION OF P METABOLITES AND COMPARTMENTATION IN SOYBEAN
NODULES AS STUDIED BY ELECTRON MICROSCOPY AND ^{31}P NMR
SPECTROSCOPY

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In vivo ^{31}P NMR spectroscopy in conjunction with electron microscopy can give complementary information concerning P distribution and metabolism in plant tissue. Soybean root nodules consist of a heterogeneous cell population. By careful separation of the cortex, central matrix and bacteroids, we have been able to obtain in vivo ^{31}P spectra of the metabolites associated with each specialized section of the nodule tissue. These results indicate that the majority of the P_i is present in the vacuole of the cortical cells whereas almost all of the P metabolites are located in the cytoplasm of the inner sphere. The intracellular pH of each characteristic cell type was also evaluated by its P_i resonance position. Electron microscopy was used to establish the distribution of host cells found within the matrix of the nodule as well as the contribution of the cortex cells to the nodule cell population. These values agree with the distribution of host cells estimated from the vacuole P_i /cytoplasmic P_i resonance intensity observed in the NMR spectra.

P0-07 RAPID PLATE ASSAY FOR HYDROLYTIC ENZYMES OF RHIZOBIUM

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It has been suggested that extracellular hydrolytic enzymes function in the penetration phase of legume root infection in Rhizobium. This study reports a simple, rapid and economical method for detecting rhizobial enzymes. Both noninfective (0402 and 0435-2) and infective (0403, Na-30, 0435 and CIAT 79) strains were tested. Drops of thick cell suspensions were placed on 1% GelRite + substrates for 1 hr., washed and flooded with developing substances: (1) 1% hexadecyltrimethyl ammonium bromide for endoglucanases (0.5% carboxymethylcellulose + 0.1% mannitol + 0.5% glycerol); (2) 6% cellulose-Azur in a two-layer system for exoglucanases; (3) 0.05% ruthenium red for pectic enzyme activity (0.1% pectin); and (4) 0.1% coomassie brilliant blue for proteolytic enzymes (0.1% gelatin). All strains were positive for cellulase. No exoglucanase activity was detectable in strains 0403, 0402, Na-30, 0435, 0433-2 or CIAT 79. Optimum pectinolytic activity was with 0.1% mannitol + 0.5% glycerol and 0.1% polygalacturonic acid or pectin as substrates. Optimum proteolytic activity with strains 0403 and 0402 was at pH 7. This method is not quantitative but provides a measurement of the potential for infectivity of Rhizobium by rapid estimate of enzyme production.

P0-08 EVALUATION OF RHIZOBIUM MELILOTI STRAINS INGENEOUS TO SONORAN DESERT SOILS BY PLASMID PROFILE AND INTRINSIC ANTIBIOTIC RESISTANCE

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Dominant strains of R. meliloti responsible for N fixation were identified from nodule isolates collected from five locations throughout the state of Arizona, which had never been inoculated. The locations were sampled in the winter of 1987 and the summer of 1988. The dominant strains ($\geq 20\%$ nodule occupancy at each sampling site) were identified through plasmid profile analysis and intrinsic antibiotic resistance patterns. Four strains which were dominant throughout the state and a commercial strain (Nitragin Co. Milwaukee, WI) were examined for their effectiveness in a Leonard jar study. All indigenous strains were as effective as the commercial strain, since there were no significant differences in shoot weight, root weight, nodule weight, acetylene reduction, and total N content among the strain treatments. The indigenous strains AZTCYJ, AZSC, and AZY have potential as inoculants for arid lands due to their effectiveness and their unique resistance to the extreme environment of arid land soils and climate. Strain AZTCYJ was widely distributed throughout the desert southwest. Strain AZY appeared to be high temperature tolerant while AZSC was salt tolerant.

PO-09

ENHANCING RHIZOBIAL COLONIZATION IN THE RHIZOSPHERE OF LEGUMES BY A SYSTEMIC FUNGICIDE

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Rhizobium does not colonize extensively in the rhizosphere of legumes even if it is inoculated on the seeds. This may result from the activities of indigenous microorganisms. A method was designed to improve the colonizing ability of rhizobia by using a basipetal systemic fungicide (aliette). R. meliloti was made resistant to this fungicide. Adding aliette with R. meliloti to alfalfa seeds significantly enhanced the colonization of roots by the test rhizobium at early stages of plant growth. A foliar spray with 2.5% aqueous solution of aliette and a R. meliloti inoculum on seeds produced a still greater stimulation of R. meliloti. The higher counts were maintained for 15 days. Plants receiving aliette and R. meliloti had larger numbers of nodules and higher yields than uninoculated alfalfa or plants inoculated with R. meliloti alone. The results suggest that such fungicides may be useful in enhancing the colonization of root-nodule bacteria in the rhizosphere and in increasing their beneficial effect on plants.

PO-10

LEVELS OF *NOD* GENE INDUCING COMPOUNDS IN UNINOCULATED AND INOCULATED *GLYCINE MAX* (L.) MERR CV. BRAGG AND ITS NODULATION MUTANTS.

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NodY::LacZ fusions were being used to compare extracts and exudates from different genotypes of *Glycine max* (L.) Merr cv. Bragg, including inoculated and uninoculated wild type, the non-nodulating mutants nod49 and nod139, and the supernodulating nts382 mutant. Preliminary results indicated no differences in the response of *Bradyrhizobium japonicum* strain ZB977 *nod* genes (as measured by β -galactosidase activity) to extracts from the different genotypes. Total HPLC profiles of fractionated extracts from these genotypes were similar in levels and composition. Daidzein has been reported to accumulated in soybean leaves after infection by some phytopathogenic bacteria. The effect of inoculation with *Bradyrhizobium* on plant-derived *nod* gene inducing compound levels is currently under investigation. Preliminary experiments showed that exudates from uninoculated wild type, and the mutants nts382, nod49 and nod139, had similar nod-gene inducing activities suggesting that neither autoregulation nor mutant phenotypes were due to an increase or decrease in levels of plant signals to the bacterial *nod* genes. It appears unlikely that alterations in flavonoid levels are responsible for the mutant phenotypes.

P0-11

RESTRICTION MAPPING AND SUBCLONING OF THE TRIFOLITOXIN PRODUCTION AND RESISTANCE GENES FROM RHIZOBIUM LEGUMINOSARUM BV. TRIFOLII T24.

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In mixed inoculum, Rhizobium leguminosarum bv. trifolii T24 limits nodulation of clover roots by other strains of R. leguminosarum bv. trifolii. The nodulation competitiveness expressed by T24 is caused by that strain's ability to produce the anti-rhizobial peptide, trifolitoxin. A recombinant plasmid, pTFX1, has been identified in a genomic library of T24 which confers trifolitoxin production in trifolitoxin-sensitive strains of Rhizobium. In this study, transposon mutagenesis and restriction analysis was used to map and subclone the trifolitoxin genes in pTFX1. A 4.4 kb region of DNA, referred to as tfx, was found to be necessary for the expression of trifolitoxin production and resistance in Rhizobium. This region was subcloned into pRK415. The resulting recombinant plasmid, pTFX2, conferred the ability to produce trifolitoxin when conjugated into trifolitoxin-sensitive strains of Rhizobium. Thus, pTFX2 possesses all of the trifolitoxin production and resistance genes. The genes for trifolitoxin resistance and production were separated by cloning a 10 kb fragment of pTFX1, containing part of tfx into pDSK519. The resulting plasmid, pTFX4, conferred trifolitoxin-resistance, but not production, in a trifolitoxin-sensitive strain of Rhizobium following conjugation.

P0-12

THE BELTSVILLE RHIZOBIUM CULTURE COLLECTION

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Since 1912 USDA-ARS has maintained a collection of the important agricultural bacterium, Rhizobium. These bacteria provide nitrogen to legumes and are an important component of the global nitrogen pool. The collection is a major germplasm resource for Rhizobium and Bradyrhizobium.

The purpose of the collection is to provide a safe and perpetual depository and collection center of Rhizobium and Bradyrhizobium cultures, which are available to users worldwide. The emphasis of the collection is on agricultural legumes, but bacterial germplasm is also available that has been isolated from wild legumes. Currently, we have 2,659 cultures isolated from 85 genera and 325 species of the Leguminosae. Also, a second edition catalog is available. We distribute between 600-800 cultures/year, and about half of the requests are from foreign countries.

In 1976, the Agency for International Development (A.I.D.) contracted with the United States Department of Agriculture to establish the "World Rhizobium study and Collection Center to complement the activities of the Beltsville Rhizobium culture collection. The establishment of this project with A.I.D. extends the activities of the collection to research scientists in lesser developed countries.

P0-13

COMPETITION FOR NODULATION OF SOYBEAN BY BRADYRHIZOBIUM JAPONICUM 123
and 138 IN MISSOURI SOIL.

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Nodules on uninoculated soybeans grown in Missouri field soil contained B. japonicum belonging to at least 8 serogroups. Serogroups c1 and 123 were present in 44-70% of all nodules, depending on plant age. Nodule occupancy by serogroup 123 changed over the growing season, but that of serogroup c1 was invariant. Smaller percentages of nodules were occupied by other serogroups, including 38-115, 76, 122, c3 and 94. Peat-formulated B. japonicum 123 and 138 inoculants were used in growth chamber competition experiments with field soil. Inoculation with strain 123 or 138 caused nodule occupancy by serogroup 123 or c1 to increase 13 and 2-fold, respectively. Inoculation with either strain substantially altered the spatial distribution of serogroups in nodules on the taproot. Dual inoculation with both strains allowed serogroup c1 to occupy 44% of the nodules and serogroup 123 to occupy 20% of the nodules. Strain 123 dominated nodule occupancy in competition with strain 138 in autoclaved field soil. It was found in 87% of all nodules. Thus a peat carrier enhances the competitiveness of strain 138, but not strain 123, in unsterile soil. However, soil sterilization results in fewer nodules being occupied by strain 138 in competition with strain 123.

P0-20

GROWTH RATE OF RHIZOSPHERE BACTERIA MEASURED BY
THE THYMIDINE METHOD

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A protocol for measuring bacterial growth in the rhizosphere based on 3Hthymidine incorporation into DNA is presented. DNA is extracted subsequent to labeling and the rate of DNA synthesis measured is equivalent to the rate of formation of cells. By taking 50 mg subsamples of soil in different regions of the root system spatial variability is registered, neither roots nor fungi is labeled only bacteria. Labeled DNA is collected on filters after precipitation following warm alcali extraction and centrifugation. Internal 14CDNA standards were extracted at an efficiency of 30-70% with 5 different soils. When bacterial production was measured on a 3 week old pot grown cucumber, the variation between 4 replicate subsamples of either soil, root tips or older roots was 20-50%, 8% growth occurred in soil without roots, 32% on root tips, and 60% on older root parts, when means were taken and comparison was made based on dry weight of adhering soil.

P0-21 CHROMOSOME AND SYMBIOTIC PLASMID DIVERSITY WITHIN A NATURALLY-OCCURRING POPULATION OF CLOVER RHIZOBIA

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Diversity in naturally-occurring populations of rhizobia has been shown by serological and protein electrophoresis techniques, but no attempts have been made to understand the genetic structure of the populations. We have used multilocus enzyme electrophoresis and restriction fragment length polymorphisms (RFLPs) around chromosomal and symbiotic plasmid DNA sequences (nif, nod, and RtRS) to characterize a field population of 150 Rhizobium leguminosarum bv. trifolii isolates. Allozyme electrophoresis revealed 18 distinct chromosome types (16 enzyme loci). There were seven lineages of either a single or cluster of chromosome types. Nevertheless, similar pSym profiles were observed in association with distinctly different chromosome types, implying that plasmid transfer had occurred. This conclusion was supported by observations of different pSym profiles in association with the same chromosome type. Overall, there was a strong correlation between pSym type and symbiotic effectiveness with two different Trifolium subterraneum cultivars.

Stability of antibiotic-resistance markers in Bacillus cereus UW85

P0-22

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Measuring rhizosphere population dynamics of the biocontrol agent Bacillus cereus UW85 requires UW85 to have a stable marker that distinguishes it from the indigenous B. cereus population. We measured the stability of spontaneous antibiotic-resistance mutations in populations of four streptomycin-resistant mutants and four neomycin-resistant mutants of UW85. One neomycin-resistant strain contained the plasmid pBC16, which carries tetracycline resistance. Stability of antibiotic resistance was examined in culture media, and in the rhizosphere of soybeans grown in a growth chamber and in the field. None of the strains lost their antibiotic resistance marker in culture media, or in the rhizosphere of 46 day-old-soybeans grown in a growth chamber. However, within 14 days after planting antibiotic resistance was lost from the population in the field. Stability of antibiotic resistance markers in the field varied with strain, experimental plot site, and environmental conditions. Differences in marker stability among strains in the field do not correlate with frequency of reversion to the wild-type phenotype or with growth rates in a complex or defined culture media.

P0-23 BACTERIAL CHARACTERISTICS IMPORTANT TO RHIZOSPHERE COMPETENCE

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A number of traits have been suggested as important contributors to successful root colonization by bacteria. A study was conducted to assess the importance of some of these traits to rhizosphere competence in a range of bacteria. Ten bacteria were isolated from soybean roots, and ten were isolated from soil. Each organism was tested for the rate and extent of growth on root exudates, adherence to the root, tolerance of low osmotic potentials, chemotactic attraction to root exudates, mobility along the root, and agglutination by root exudates. Data were evaluated to determine if organisms isolated from the root were significantly different from those isolated from soil in any of the traits. The data show that root organisms have greater mobility along the root than do soil organisms. The results of tests of the other characteristics will be presented.

P0-24 APPLICATION OF HIERARCHICAL THEORY TO MICROBIAL FAUNAL INTERACTIONS IN TWO CONTRASTING SOILS

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Soil ecologists study components of the C cycle and face the challenge of integrating observation sets obtained at different levels to ecosystem processes. The hierarchical theory provides a conceptual framework to integrate these processes in order to understand emergent properties. We have applied these concepts to understand C flow in two contrasting soils (Typic Cryoboroll and Typic Cryoboralf) cropped to barley. Observation sets included soluble C, microbial C, respired C, soil organic C, and protozoa, nematode, acari and collembola populations. The levels of organization were linked through phenomena (hypotheses) and were useful to interpret differences in C cycling in the two soils.

P0-25

**A ROOT TIP ENRICHMENT TECHNIQUE TO SELECT BACTERIA WITH
HIGH ROOT COLONIZING ABILITY**

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Roots are colonized primarily by bacteria originating from colonies in the soil with which the root makes contact. If these chance colonists respond to root exudate, colonies develop and occupy those niches which favour proliferation e.g. root cell junctions, moribund cells, mucigel. Prior occupancy of roots by native soil bacteria makes it difficult for organisms introduced via seed coating to become established. In our study, we hypothesized that bacteria capable of utilizing polysaccharides exuded with root cap cells as the root grows through soil may be more effective root colonizers than bacteria isolated from the rhizospheres from older plants. We enriched three soils with different cropping histories by germinating wheat seeds in the soils, and when the roots averaged 1 cm length, cutting them off and leaving them in the soil. This was repeated 7 times over four weeks. Wheat seedlings were grown in the enriched and control soils and, after 7 days, counts of bacteria made on roots and adhering soil with different agar media. Enrichment with root tips led to a 3 to 6-fold increase in the numbers of total bacteria and a 15 to 20 fold increase in numbers of pseudomonads in the rhizospheres of wheat grown in the two soils from continuous wheat but only 2-fold and 11-fold increases in total bacteria and pseudomonads respectively in soil from a field in which different crops and pastures were rotated. Amongst the pseudomonads isolated from the rhizosphere of wheat roots grown in enriched soils were a number of isolates which could keep pace with the root tip for 10 days in unsterilized soil without any water movement down the soil. These bacteria were more effective root colonizers than *P. fluorescens* strain 2-79 (Weller and Cook, 1983) which was used as a standard.

P0-26

**IMPROVED RHIZOSPHERE COMPETENCE OF TRICHODERMA HARZIANUM
BY PROTOPLAST FUSION**

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Protoplast fusion between 2 auxotrophic mutants of Trichoderma harzianum (T12 and T95) resulted in a wide variety of prototrophic progeny. One of the strains from protoplast fusion (1295-22) showed better biocontrol activity than the parental strains. Here we report quantitative data on the improved rhizosphere competence of this strain compared with its parents. Seeds of cotton and sweet corn were treated with conidia of these strains and planted in a soil with a constant moisture content (15%; v/w). The tested strains could be isolated from the rhizosphere of most portions of corn roots 20-22 cm long. T95 colonized mainly the upper and the lower parts of the roots while most of T12 population densities were found primarily on the upper third of the root. However, the protoplast fusion progeny, 1295-22, colonized the entire root. T95 and T12 along the lower half of corn root were obtained mainly from the rhizosphere soil but not from the rhizoplane. Conversely, strain 1295-22 colonized both the rhizosphere soil and the rhizoplane. Although, population densities of these strains were, in general, lower in cotton rhizosphere than with corn, 1295-22 colonized more root segments than the parental types.

P0-27

GROWTH OF GENETICALLY-ALTERED *PSEUDOMONAS SOLANACEARUM* IN SOIL AND RHIZOSPHEREJ.W. Williamson¹, P.G. Hartel¹, and M.A. Schell²Depts. of Agronomy¹ and of Microbiology², Univ. of Georgia, Athens, GA 30602

The effect of the genetic alteration of the *egl* gene encoding for a β -1,4-endoglucanase on the growth of *Pseudomonas solanacearum* in freshly-moistened soil and in the rhizospheres of tomato (*Lycopersicon esculentum*), common purslane (*Portulaca oleracea*), and pearl millet (*Pennisetum glaucum*) was determined. Endoglucanase production was either eliminated by transposon (Tn5) insertion mutagenesis or enhanced two-fold by increasing the *egl* copy number with a recombinant plasmid (pLAFR3). These genetically-altered strains had increased generation times in soil solutions extracted from the two soils, and this was significantly correlated ($r = -0.97$) with their ability to grow in the same, freshly-moistened soils. None of the strains, including the wild type, grew in the rhizosphere of tomato, common purslane, or pearl millet. Under conditions of soil inoculation, the genetically-altered strains did not wilt tomato seedlings significantly faster than the wild-type strain. The data suggest that genetic alterations have an effect on the growth of *P. solanacearum* in soil and that the endoglucanase gene is of little importance in the root infection process.

P0-28

GROWTH AND SURVIVAL OF GENETICALLY-ALTERED *PSEUDOMONAS AERUGINOSA* AND *PSEUDOMONAS PUTIDA* IN SOIL AND IN RHIZOSPHEREK.-H. A. Yeung¹, M. A. Schell¹, and P. G. Hartel²Depts. of Microbiology¹ and Agronomy², Univ. of Georgia, Athens, GA 30602

The effect of the addition of the *pglA* gene encoding for an endopolygalacturonase from *Pseudomonas solanacearum* on the growth and survival of *Pseudomonas aeruginosa* and *Pseudomonas putida* in soil and rhizosphere was investigated. The *pglA* gene on a 30 kb *P. solanacearum* DNA fragment in the 20 kb cosmid vector pLAFR3 (now called pJE8) and the vector alone were conjugally transferred into both *P. aeruginosa* and *P. putida*. Both strains carrying pJE8 produced high levels of intracellular and extracellular polygalacturonase. Under growth conditions in freshly-moistened potting soil, the generation times and final cell densities of the two genetically-altered strains with either pLAFR3 or pJE8 were not significantly different from the wild-type strains. At a density of 10^5 colony-forming units g⁻¹ of soil, all strains either remained at the initial inoculation density or declined in density in the rhizosphere of tomato (*Lycopersicon esculentum* Mill. cv. Marion). Survival studies are currently in progress. The results suggest that polygalacturonase production does not result in increased bacterial fitness.

P0-30 PHOTOREGULATION OF ROOT:SHOOT RATIO IN SOYBEAN SEEDLINGS

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The relative growth of root and shoot maintains remarkable constancy under steady-state conditions yet varies in response to changes in environment (light, moisture, nutrients, atmospheric pollutants, etc.) or developmental status. The root:shoot ratio appears to be an important aspect of overall plant growth regulation and may be influenced by so-called "functional equilibria". For example, reduced photoassimilation in the shade could limit roots more than shoots as a result of carbohydrate availability. However, it is shown here that root:shoot ratio is strongly affected by shade-responsive photomorphogenetic photoreceptor systems (chiefly blue light photoreceptors but also phytochrome). This regulation is apparent early in seedling development (i.e., emergence) and can be divorced from alterations in net seedling growth by appropriate light treatments. Thus, root:shoot ratio clearly involves more than relative inputs of water, nutrients and carbohydrate. Spectral quality-related alterations in partitioning of growth to roots may affect interactions between the plant and soil microorganisms, plant competition related to differential root growth, ability to engage in N-fixation, ability to obtain water and nutrients as well as harvest index.

P0-31 RESPONSE OF SYMBIOTIC SOYBEANS TO ACIDIFIED SOIL

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Soybean plants (Glycine max L. cv. Essex) were grown outdoors in pots containing N-deficient Maury soil (pH 6.3) or similar soil amended with sufficient $\text{Al}_2(\text{SO}_4)_3$ or S_2 to give soil pH values of 4.8 and 4.6, respectively, and water-extractable Al levels of 45 and 26 μM , respectively. Other treatments consisted of the addition of inorganic N or inoculation with commercial or locally-isolated Bradyrhizobium japonicum. Acidification did not significantly reduce shoot or root dry weights of plants receiving inorganic N, but acidification significantly reduced shoot and root dry weights, nodule dry weights and number, and foliar N content of inoculated plants. Thus, under acid soil conditions the adversely-affected symbiosis appeared to be the primary cause of reduced plant growth. Inoculated plants grown in S_2 -amended soil were significantly smaller than those grown in $\text{Al}_2(\text{SO}_4)_3$ -amended soil which was less acidic but contained more soluble Al than the former soil. Thus, acidity itself appeared to be more detrimental to plant growth than Al toxicity. Addition of Mo to inoculated plants was not beneficial.

P0-32

THE OCCURRENCE OF CLUSTER ROOTS IN ACTINORHIZAL PLANTS

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First termed as 'proteoid roots' because of their common occurrence in the Proteaceae, cluster roots are lateral root proliferations along an elongating root axis. They have also been reported in several species of leguminous plants. Our studies extend their occurrence to the nitrogen-fixing actinorhizal plants. The mutual exclusion of mycorrhizal associations in cluster-rooted plants and the induction of cluster roots by low phosphate levels both suggest a role in facilitating phosphorus acquisition by plants due to increased surface area and absorbing ability. In water culture we have observed cluster roots in Comptonia peregrina, Myrica cerifera, Myrica gale, Myrica pensylvanica (Myricaceae) and Gymnostoma papuanum (Casuarinaceae) in solutions either lacking in phosphate or containing no more than 16ppm phosphorus. The elicitation of cluster root formation in these actinorhizal plants by low P levels will be presented.

P0-33

A SOIL BIOTRON FOR EXPERIMENTAL STUDIES OF SOIL BIOTA

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Spatial and temporal relationships of roots, microorganisms, and invertebrates can be observed directly and manipulated in the soil biotron constructed at the University of Michigan Biological Station in northern, lower Michigan. Soil was replaced around the windows in July 1987, and during the 1988 growing season the 1.5 m distance between the windows and the native forest soil has been undergoing colonization. Invertebrates were censused in transects along the windows in 1988; they were not distributed randomly throughout the soil. Over half of the collembola, oribatid, and prostigmatid individuals were on roots. All of the protura and pauropoda were on roots. Pauropods were only observed on root tips, but individuals of other groups were not shown to occur on specific root zones.

P0-34

WATER UPTAKE PATTERNS IN LOBLOLLY PINES AS SEEN WITH MAGNETIC RESONANCE MICROSCOPY

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Proton magnetic resonance microscopy was used to study water uptake patterns on containerized loblolly pines (*Pinus taeda* L.). Glass NMR tubes 5mm in diameter were filled with acid washed sand <0.5mm diameter. Water was added at 0, 5, 10, 15, and 20% (w/w). Signal intensity from protons (¹H) increased with water content. Containerized loblolly pine seedlings were transplanted into 3 cm diameter containers holding a similar sand substrate. Sand around the woody taproot was the area of most rapid water loss compared lateral roots and mycorrhizal short roots. Regions of water loss around taproots also appeared larger compared to regions around smaller roots. These observations suggest large diameter woody roots are one of the major locations for water uptake under the conditions of this study.

P0-35

DISTRIBUTION OF ASSIMILATED CARBON WITHIN THE PLANT AND RHIZOSPHERE OF LOLIUM PERENNE : COMPARISON OF FIELD AND LABORATORY GROWN PLANTS

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A comparison between carbon lost from rye-grass roots from laboratory and field grown plants was made in an investigation of factors affecting rhizosphere carbon-flow under natural conditions. Laboratory grown plants matured more rapidly, and there was a decrease in investment of ¹⁴C-CO₂ pulse label in the root biomass when compared to plants grown in the field. As both field and laboratory grown plants developed, less carbon was translocated below ground as a percentage of net assimilated pulse label. Loss of label from roots and the ability of the plants to assimilate label (expressed in kBq g⁻¹ plant biomass) also decreased as plants developed. A number of environmental factors affecting root exudation such as temperature, soil water potential, soil microflora, soil pH and anaerobism were investigated to interpret the pattern of carbon-flow from perennial rye-grass under natural conditions.

P0-36 TEMPERATURE DEPENDENCE OF THE TONOPLAST AND PLASMA MEMBRANE
H⁺-ATPASES FROM MAIZE ROOTS

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The effects of temperature on the activities associated with the H⁺-ATPases from tonoplast and plasma membranes were determined. From 5 to 45 C, initial rates of ATP hydrolysis by both pumps obeyed a simple Arrhenius model with an activation energy of 14 kcal/mol. In addition, the K_m for ATP hydrolysis was relatively insensitive to temperature changes with both pumps. However, proton transport by the tonoplast ATPase was affected by temperature quite differently. Over the temperature range of 5 to 45 C, initial rates of proton transport showed a bell-shaped temperature dependence with a maximum between 25 and 30 C. Additionally, the ATP dependence of the proton transport activity varied with temperature. The K_m for ATP increased with increasing temperature. Detailed kinetic analysis of proton pumping indicated that increases in proton leakage during the pumping stage constituted a major reason for the decreased proton transport at higher temperatures.

P0-40 CHARACTERIZATION OF CULTIVAR SPECIFIC GROWTH PROMOTION OF
SPRING WHEAT BY BACILLUS SP.

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We have shown previously that six strains of Bacillus sp. isolated from the rhizosphere of spring wheat cv Katepwa were able to promote root growth of Katepwa but not of the parental cultivar Neepawa under sterile conditions in test tubes. Further studies with strains, 5A1 or 3A have shown that the growth promotion can be demonstrated under a variety of growth conditions, in sterile Leonard jars, non-sterile pots and in the field. A heat labile factor may be involved in the mode of action as autoclaving 5A1 cells inhibited growth promotion but addition of supernatant was as effective as whole cells. IAA was detected in the supernatant using GCMS. In the field, inoculation of Katepwa with 5A1 increased shoot weight and tiller number by 38% (P=0.10) and 79% (P=0.05), respectively relative to controls 70 days after planting. Shoot weight and tiller number in Neepawa were not affected by inoculation with 5A1 or 3A.

P0-41 EFFECT OF *BACILLUS* STRAINS ON GROWTH OF PINE
(PINUS CONTORTA DOUGL.), SPRUCE (*PICEA GLAUCA*
VOSS.) AND DOUGLAS FIR (*PSEUDOTSUGA MENZIESII*
(MIRB.) FRANCO)

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Inoculation of lodgepole pine and white spruce with two *Bacillus polymyxa* strains was shown to promote growth. Strain L6 caused significant increases in lodgepole pine shoot and root dry weight, root surface area, and root collar diameter after 8 weeks growth from seed, but these effects disappeared by 16 weeks. When 1-0 containerized stock was inoculated, shoot growth was increased, but root weight increases were not significant. Strain L5 significantly increased root surface area of both lodgepole pine and white spruce after 12 weeks growth. Dry weight gains were not significant. As with L6, differences among treatments declined to non-significance by 16 weeks. Neither isolate affected growth of Douglas fir.

P0-42 RHIZOSPHERE EFFECT ON SOIL ORGANIC MATTER
 DECOMPOSITION

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In our study, ¹⁴C-labelled rye straw was incubated in fertilized or unfertilized soil with or without plants for 49 days under semi-controlled conditions. Our objective was to study the rhizosphere effect on soil organic matter mineralization under different soil mineral nutrient conditions. The planted treatment had a higher ¹⁴CO₂ loss and a higher efficiency of ¹⁴C-labelled material utilization by the microorganisms. Fertilization decreased ¹⁴CO₂ loss. Percent ¹⁴C in microbial biomass was positively correlated with percent ¹⁴C respired ($P<0.001$). Total microbial biomass, ¹⁴C remaining, and ¹⁴C-labelled microbial biomass of the rhizosphere soil were higher compared to the bulk soil. The rhizosphere soil in the fertilized treatment had a higher percent labelled microbial biomass and a lower total microbial biomass than that in the unfertilized treatment. This suggests that fertilization reduces the rhizosphere effect.

PO-43

INTERACTION BETWEEN AZOSPIRILLUM AND THE HOST PLANT:
MODELS AND RESEARCH APPROACHES

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Positive effects of Azospirillum inoculations on plants have been described. However, the stimulating effects of this bacterium on the plant do not seem to be confined to the N₂ fixation contribution, but seem to involve the influence of phytohormones produced by the bacteria or to stimulate the plant to produce growth substances in the root. Possible interactions between plant and microorganism are: 1) Direct contribution to the plant of N₂ fixed by the Azospirillum, 2) Indirect contribution of N₂ fixed to the plant through the increase of the soil N pool, 3) Plant growth substances produced by the bacterium directly influencing the root growth, 4) Plant stimulation to produce hormonal compounds, and 5) Indirect positive effects of the Azospirillum on the plant, like protection from pathogens, siderophore production, etc. Fragmentary and some times unrigorous proof has been given to confirm these different theories concerning the nature of the relationships which occur between cereals and forage grasses and Azospirillum. Different strategies can be adapted to approach these problems. A protocol of experimental approaches will be discussed. Particular emphasis should be given to the definition of the initial steps of plant and microorganism contact.

PO-44

SULFUR OXIDIZING MICROORGANISMS FOR GROWTH
PROMOTION OF CANOLA

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Canola has a high sulfur requirement during vegetative growth and exhibits symptoms of sulfur deficiency when grown on Saskatchewan soils low in plant available sulfur. Elemental sulfur is frequently used as a fertilizer to alleviate this deficiency. The potential of sulfur oxidizing microorganisms to enhance the growth of canola in sulfur fertilized soils was assessed. Sulfur oxidizing bacteria and fungi were isolated from the rhizosphere and rhizoplane of canola grown in four soils under growth chamber conditions. Of 273 bacterial isolates, 245 (89.7%) oxidized sulfur to thiosulfate or tetrathionate in vitro, and 133 (48.7%) oxidized sulfur to sulfate; all 70 fungal isolates oxidized sulfur to sulfate. Eighteen isolates demonstrating the highest in vitro sulfur oxidation were tested as seed inoculants under growth chamber conditions. Thirteen isolates increased canola leaf size measured at the bud stage of growth, and seven isolates increased root and pod dry weights at maturity. Three isolates inhibited the growth of the canola fungal pathogens, Rhizoctonia solani AG2-1, R. solani AG4 and Leptosphaeria maculans - Leroy. One of these isolates was antagonistic towards both R. solani strains and one inhibited the growth of R. solani AG2-1 and L. maculans - Leroy. Thus some sulfur oxidizing isolates appear to stimulate canola growth due to the enhancement of sulfur uptake, whereas in some cases antibiosis towards canola pathogens may also be involved.

PO-45

MICROBE ENHANCED P UPTAKE BY CORN UNDER NO TILL AND CONVENTIONAL TILL

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This study was made to determine the contribution of microbial activity to P uptake by corn, Zea mays L., under field conditions. The objective was to measure, in the presence and absence of microbial activity, P uptake and growth of corn in plots that differed in soil P fertility and tillage management. Plots under no till and conventional till management were fumigated and planted to corn. The rate of P uptake, plant growth, and grain yield were monitored. In the fumigated low-soil P plots P uptake rate was one-fifth to one-half those of the corresponding check plots under both tillage managements. These lowered rates of P uptake were accompanied by lowered growth rates and lowered grain yields. In field plots with moderate levels of soil P, fumigation lowered P uptake rates of conventional tilled corn but not in the no till managed corn. At high soil P fertility, fumigation had no effect on P uptake and growth under either tillage management. The study showed that P uptake rate is significantly increased by apparent microbial activity. This activity allowed an adequate growth rate under marginally low soil P conditions.

PO-46

INFLUENCE OF NITROGEN FERTILIZATION ON PHOTOSYNTHATE DISTRIBUTION AND UTILIZATION BY RHIZOSPHERE MICROORGANISMS IN WHEAT.

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The transport of photosynthates from the leaves to the rhizosphere of wheat and incorporation in the soil microbial biomass was investigated by growing plants in a continuously ¹⁴C-labelled atmosphere. Two nitrogen levels in soil were applied. At a high nitrogen level significantly more ¹⁴C was released by the roots indicated by higher percentages of the assimilated ¹⁴C in root-soil respiration and soil residue. The total root biomasses did not differ significantly between the two nitrogen levels. However, the amount of labelled microbial biomass in the soil was significantly higher at the high nitrogen level. The bacterial counts in the rhizosphere soil were also higher at high nitrogen level. It would appear that most of the increase in microbial biomass resulted from a higher exudation rate at the higher nitrogen level. However, the differences in microbial biomass were larger than the differences in release in carbon from the roots. This indicated that the root exudates were more efficiently used at the high nitrogen level. While high nitrogen treatment stimulated the decomposition and microbial utilization of root-released materials it appeared to have an opposite effect on soil organic matter since the rate of respiration of unlabelled carbon from soil decreased.

PECTIC ENZYMES OF AZOSPIRILLUM BRASILENSE

P0-47

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The production and activity of pectic enzymes by Azospirillum brasiliense (strain C.A. 10), a diazotroph isolated from the rhizosphere of rice, was studied because this organism colonizes the histosphere of rice roots via entry through the middle lamella. A. brasiliense produced adaptively exo-polygalacturonase (Exo-PG), pectin transeliminase (PTE) and pectic acid transeliminase (PATE) in vitro with either pectin or pectic acid as sole sources of carbon. The production of endo-polygalacturonoase (Endo-PG) was found in trace amounts and that of pectin methyl esterase (PME) was nil. The regulatory role of pH levels (5.5 to 8.5) on the production and activity of pectic enzymes was studied. Growth was positively correlated with Exo-PG production. The production and activity of Exo-PG were found to decrease with increase in pH levels from 5.5 to 7.0. Further, the production of transeliminases was found to be at pH 6.5 to 7.0. Since the optimum activity of the transeliminases is at pH 8.7, we conclude that the hydrolases play a relatively more significant role than the transeliminases in the entry of this diazotroph into the histosphere of rice roots.

P0-50

EXPOSURE OF TIMOTHY GRASS TO SO₂ AND ITS SUBSEQUENT EFFECT ON
VAM ROOT INFECTIVITY

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Two experiments were conducted to test the indirect effects of SO₂ (sulphur dioxide) fumigation on the ability of VAM to infect and proliferate in the roots of Timothy (Phleum pratense L.) which had been continuously exposed to 0.05 - 0.07 ppm SO₂. Timothy seedlings (6 day-old) were planted into 65 ml syringe barrels which had been filled with field soil. The field soil contained known sources of VAM inoculum. The seedlings were then placed into either an SO₂ exposure chamber or control chamber. The chambers were designed in such a way that only the shoots of plants were exposed to SO₂. In the first experiment, seedlings were continuously exposed to SO₂ for 6 wk. In the second experiment, seedlings were allowed to grow under control (non-SO₂) conditions for 3 wk before being continuously exposed to SO₂ for the remaining 3 wk. The results showed that SO₂ fumigation of Timothy shoots inhibits the ability of VAM to infect and proliferate within host roots. These experiments also showed that VAM infection has a profound effect on tillering, leaf area, and shoot and root dry weight, regardless of SO₂ exposure.

P0-51

PROMOTION OF MAIZE GROWTH BY LEGUME SOIL FACTORS

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Maize (cv W64A x W182E) was grown in a low nutrient sandy loam in green-house conditions (25°C, 16h/8h light/dark cycle). Inoculation with legume soils (4.1% v/v) resulted in a 3-4 fold increase in shoot growth relative to controls in 5-8 weeks. Inoculum sterilization (γ -irradiation [3.6 Mrad] or autoclaving) eliminated this response. Treatment of the sandy loam with the fungicides benomyl or PCNB also eliminated the response whereas metalaxyl, etridiazol and the bactericide streptomycin has no effect. These results and the elimination of both the growth response and VAM infection by low level γ -irradiation (100 Krad) of the inoculum suggests a role for VAM fungi in the shoot growth enhancements. Inoculation with maize soils gave no shoot growth response. This project was funded by a strategic grant from N.S.E.R.C., Canada.

P0-52

CORRELATION OF INFECTIVITY OF CITRUS SPECIES WITH THEIR
MYCORRHIZAL DEPENDENCY

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To evaluate vesicular-arbuscular mycorrhizal (VAM) infectivity of roots of known age in comparison to undisturbed roots, soil cores of 450-cm³ volume were extracted from beneath the canopy of mature trees in a well-fertilized rootstock trial. Soil was replaced after dry sieving to remove roots and mix VAM propagules. Extra- (EM) and intraradical (IM) colonization, root length, biomass and diameter were evaluated 5, 10 and 19 wks later. In disturbed soil, EM colonization increased up to 10 wks and approached that of undisturbed soil by 19 wks. IM colonization increased up to 19 wks and was less than that in undisturbed soil. Rootstocks varied significantly ($P < 0.05$) in EM colonization after 10 wks and IM colonization after 19 wks as follows: Volkamer lemon > rough lemon = sour orange = Cleopatra mandarin > Swingle citrumelo > trifoliate orange. Infectivity of new roots was not related to root diameter or root extension rate of the rootstocks. Rootstock infectivity was highly correlated ($r = 0.98-0.99$; $n = 3-4$) with previously determined mycorrhizal dependency (MD = VAM dry wt./NM dry wt.) in 3 greenhouse trials in a sterilized, P-deficient soil. Therefore, MD and infectivity of citrus species are not related to root diameter and extension rate but apparently to other genetically determined attributes.

P0-53

VARIATION IN VA MYCORRHIZAL STRAIN INTERACTIONS
WITH RHIZOBIUM ON PIGEON PEAD. C. Ianson¹ and R. G. Linderman²

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Pigeon pea plants, inoculated with an effective strain of Rhizobium and each of seven different strains of VA mycorrhizal (VAM) fungi (Glomus and Gigaspora), exhibited strikingly different degrees of growth enhancement after 12 wks growth. Growth increases were independent of tissue P levels, and highly correlated to number and size of nodules formed and thus N fixed. The non-VAM control and two VAM treatments formed very few nodules, while other VAM plants formed 75-100 nodules. Tolerance to soil drought was enhanced by some VAM strains more than others, but the drought reaction was not correlated with the nodulation response. These results indicate the requirement for inoculation of pigeon pea (and probably all legumes) with Rhizobium plus a compatible VAM strain for optimization of nitrogen fixation and growth as well as drought tolerance.

P0-54

HISTOCHEMICAL ASSESSMENT OF FUNGAL MASS AND BIOTIC ACTIVITY IN ECTOMYCORRHIZAL ROOTS, RHIZOSPHERE SOIL AND NON-RHIZOSPHERE SOIL.

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Fungal mass and biotic activity of soil and Pinus contorta roots ectomycorrhizal with Hebeloma crustuliniforme were estimated by quantification of ergosterol and activity of ornithine decarboxylase. Over a 12 week period, roots harbored up to 70 times more mycelia per gram than soil. The rhizosphere contained up to 6 times more mycelia than non-rhizosphere soil. Root mycelia content declined over time due to dilution of intramatrical mycelia into structural tissue, while soil mycelia content remained constant. Although root activity was 45 times that of soils, the three components exhibited declines over time, likely due to root maturation and soil nutrient immobilization. The relative contribution of fungi to enzyme activity differed among components, with intramatrical hyphae being a minor contributor in roots. Non-rhizosphere soil activity was dominated by extramatrical hyphae to a greater extent than in the rhizosphere where other microorganisms were sustained. Relationships among parameters in the three components were described by correlation analyses. Rhizosphere activity was correlated to root activity and rhizosphere mycelia content but not to root mycelia content. Mycelia content of the rhizosphere was also correlated to mycelia content of non-rhizosphere soil.

P0-55

GENETIC TRANSFORMATION OF PROTOPLASTS FROM AN ECTOMYCORRHIZAL FUNGUS

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Protoplasts of the ectomycorrhizal basidiomycete, Laccaria laccata, were transformed at frequencies of 5-50 transformants per ug of DNA. Transformation was based on selection for resistance to hygromycin B using the Escherichia coli hygromycin phosphotransferase (hph) gene bracketed by an Aspergillus nidulans glyceraldehyde-3-phosphate dehydrogenase (gpd) promoter and the transcription terminator region of the A. nidulans tryptophan synthetase (trpC) gene. Southern blot hybridization revealed that transformants of L. laccata integrated vector sequences involving one or more insertional events. These results provide the first evidence for transformation of a mycorrhizal fungus and further demonstrate the ability of expression signals of ascomycetous origin to function in a basidiomycete. The potential to improve ectomycorrhizal symbiosis through transgenic manipulation of the fungal component can now be realized, provided specific genes beneficial to this symbiosis can be identified. Successful transformation of L. laccata also portends the ability to transform other species of basidiomycetes, especially cultivated mushroom species.

P0-56

OZONE-INDUCED ALTERATION OF BIOMASS ALLOCATION AND NDFA IN THE
LEGUMINOUS PLANT - BNF SYSTEM

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Ozone (O_3), a photochemical air pollutant, suppresses availability of carbon to roots. Thus, O_3 may alter symbiotic interactions among leguminous plants, rhizobia, and mycorrhizal fungi. Responses of the three-organism system to O_3 were examined in a greenhouse experiment. Germinating seeds of subterraneum clover (*Trifolium subterraneum*) were inoculated with *Rhizobium leguminosarum* ("R"), an endomycorrhizal fungus (*Gigaspora margarita*, "G"), both ("R+G") or neither ("N") and transplanted into 10-cm-diam pots containing a low-P, acidic, sandy loam soil amended with ^{15}N -labeled urea. Plants were grown for 16 da and were exposed during the next 12 wk (Mon through Fri, 6 hr/da) to O_3 (0, 0.05, 0.10, or 0.15 $\mu\text{l/l}$ of air) in greenhouse chambers. Shoot dry weight (SDW) was not affected by O_3 ; inoculation with R or R+G stimulated SDW by 221 or 275%, respectively, compared to N plants. Root dry weight (RDW) also was stimulated by inoculation with R or R+G. Ozone suppressed RDW of all plants, but this effect was stronger for plants inoculated with R or R+G than for those that did not receive R (i.e., N or G). Thus, shoot:root ratios increased with increasing O_3 . Nitrogen derived from the air (Ndfa) and from the soil (Ndfs) were quantified by the isotope dilution technique. The ratio Ndfa:Ndfs decreased with increasing O_3 in plants inoculated with either R or R+G. Inoculation with R+G did not alter total Ndfa (mg/plant) compared to inoculation with R only. However, inoculation with R+G did increase total Ndfs, resulting in plants with greater total nitrogen content but less %Ndfa than plants inoculated with R only.

P0-57

STRATEGY FOR INOCULATING NURSERY-GROWN SEA OATS
WITH VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI.

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Beach replenishment sand is devoid of VAM fungi. Colonization of sea oats (*Uniola paniculata* L.) with VAM fungi has been shown to significantly increase their growth both in greenhouse and beach studies. For example, in one beach study nursery-inoculated sea oats were 219% larger than noninoculated plants 19 mo after outplanting. Inoculation with an effective VAM fungus under commercial fertility and pesticide regimes resulted in colonized plants. Eight weeks after inoculation colonization was close to 30% even with regular applications of soluble fertilizer. In an inoculum-dilution study colonization resulted even at a rate of 1:64. The ultimate aim is for the commercial grower to produce and use inoculum of VAM fungi in the production of sea oats for outplanting on replenishment sand. Aeroponic culture of the effective VAM fungus (*Glomus macrocarpum* Tul. and Tul., isolate S328) has been successful and we are now testing the transfer of this inoculum-production technology to the grower. Testing of inoculum chopping and drying regimes, dilutions and shelf life should reveal an acceptable method for use in the commercial sea oat nursery.

P0-58

A MISTING APPARATUS FOR STUDYING PLANT-MICROBE INTERACTIONS AND NUTRIENT UTILIZATION.

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Determining the effects of field and cultural practices and of competitive microbial interactions on the populations of and infections by different VAM fungi has not been possible because methods for their study have not been available. Thus, to enhance our biochemical and genetic studies of VAM fungi, a misting (fogging) apparatus was designed for culturing VAM infected plants. Exceptionally clean VAM hyphae and spores were produced in a short time period free from plant and soil debris. Continuous harvesting was possible yielding a reliable source of biomass for immunological, biochemical, and molecular genetic studies. Leek, corn, and other desirable plants adapted to the nutrient fogging technique with prolific root production. Numerous fogging and misting nozzles were evaluated for specific applications (eg. root hair production, micronutrient studies, VAM biomass, etc.). Enhancements and modifications to the apparatus and nutrient composition will be presented.

P0-59

INCREASING VA-MYCORRHIZATION WITH APPLICATIONS OF RHIZOSPHERE BACTERIA

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Bacteria were isolated from the rhizosphere of mycorrhized plants. Several of these isolates could cause an enhancement of VAM infection when applied to the root systems of different plants. The effect was independent from the VAM isolates used. A Bacillus cereus isolate showed the best and most constant activity. It was possible to inoculate both organisms together on the same particles of an expanded clay. The increase of VAM infection appeared in the early phase of mycorrhization. In plants with an intense mycorrhizal colonization of the root system the difference disappeared in late phases. The pot culture experiments were done in the greenhouse with sand as substrate. When the sand was mixed with more than 30% commercial compost, bacteria could not increase VAM infection.

P0-60

SPECIFIC INTERACTIONS OF BRADYRHIZOBIUM AND FOUR
VA-MYCORRHIZAL ISOLATES IN SOYBEAN

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Seven soybean cultivars were inoculated simultaneously with Bradyrhizobium and four selected VAM isolates to test specific interactions and symbiotic efficiency of the microsymbionts. Plant were intensely mycorrhized. One of four VAM isolates showed a lower mycorrhization and biological activity in all cultivars. In six of seven cultivars at least one VAM isolate caused a significant increase of nodule formation. The extent of this effect depended on the VAM isolate. Simultaneous inoculation with Bradyrhizobium and VAM significantly promoted shoot growth in four of seven cultivars. The increase depended on the combination of Symbionts. There was no correlation of growth promotion with increased nodulation.

P0-61

MYCORRHIZAL FUNGI INCREASE YIELDS OF WINTER WHEAT

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Several species of mycorrhizal fungi grown in pot cultures and a sterile control were banded under winter wheat seed planted into fumigated (methyl bromide) and nonfumigated plots at Sidney, Nebraska. Mycorrhizal fungi were absent from fumigated plots. Nonfumigated plots had natural populations of mycorrhizal fungi. Introduced mycorrhizal fungi caused significant increases in wheat yields in only one of three harvests. Nonfumigated plots had significantly greater yields than fumigated plots for two of three harvests. The greater yields in nonfumigated plots were caused by increased wheat seed size (weight per 1000 seeds). Mycorrhizal colonization levels of wheat roots by introduced fungi were very low compared with colonization levels in nonfumigated plots. In a greenhouse study, wheat plants colonized by a mycorrhizal fungus produced larger seeds (weight per 1000 seeds) than nonmycorrhizal controls.

P0-62

**RESPONSE OF TWO ACACIA SPECIES TO DROUGHT AND
INOCULATION WITH AN ECTOMYCORRHIZAL FUNGUS**O. Osonubi¹ and K. Mulongoy²¹University of Ibadan, ²International Institute of Tropical Agriculture Ibadan, Nigeria

Inoculation of *Acacia auriculiformis* with *Boletus suillus* in sterile soil increased P uptake and dry matter production of both daily- and weekly-watered plants by 41 - 68%. Significant (at $P < 0.05$) correlations between shoot P content and root dry weight ($r = 0.545$) or plant dry weight ($r = 0.539$) suggested that enhanced biomass accumulation was related to increased P uptake. Inoculation of *A. mangium* with *B. suillus* also increased P uptake and plant dry matter but only for daily-watered plants. Correlations between shoot P content and plant or root dry weight were poor. Shoot N content of both *Acacia* species was not affected by inoculation with mycorrhiza. Data on leaf area and stomatal conductance showed that inoculation with the ectomycorrhizal fungus increased drought tolerance of *A. auriculiformis* but not of *A. mangium*.

P0-70

THE EFFECT OF A FLUORESCENT PIGMENT PRODUCING-
RHIZOBIUM ON THE SEVERITY OF RHIZOCTONIA SOLANI SEED
 AND ROOT ROT OF PHASEOLUS VULGARIS.

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Thirty-two strains of Rhizobium leguminosarum biovar phaseoli (R.l.p.) were examined in the laboratory for their ability to affect the growth of 6 strains of Rhizoctonia solani, the causal agent of seed and root decay of dry bean (Phaseolus vulgaris). Five strains of R.l.p. were capable of reducing the pathogen's growth by 20-50%. Three of the five antagonistic strains produced a fluorescent pigment on King's Medium B (KMB), while none of the strains having little or no effect on fungal biomass production produced pigment. Strain 527-127K17 was selected for study in the greenhouse. The number of surviving plants, disease severity, and top dry weights were determined to assess the impact of R.l.p. on disease expression in the plant. Significant disease reduction was observed with R.l.p. 527-127K17 in two of three greenhouse tests. Production of a fluorescent pigment on KMB by plant-growth-promoting pseudomonads is used to detect siderophore production. It is possible that the mechanism of R.l.p. 527-127K17 inhibition of the pathogenic fungus is a result of siderophore production. If rhizobia produce siderophores and have the ability to decrease the incidence and/or severity of seed and root rot, then it will be useful to screen seed inoculants for siderophore production.

P0-71

ROLE OF AMMONIA AND CALCIUM IN LYSIS OF ZOOSPORES
 OF PHYTOPHTHORA spp. BY CULTURE FILTRATE OF BACILLUS
CEREUS STRAIN UW85

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Cell-free culture filtrate of the biological control agent Bacillus cereus strain UW85 lyses zoospores of both Phytophthora cactorum and P. megasperma f. sp. medicaginis *in vitro*. Growth of UW85 causes an increase in ammonia and pH and a decrease in calcium in casamino acid medium, resulting in a ratio of $pCa^{2+}:pNH_3$ of 1.4. Zoospores lyse in solutions with $pCa^{2+}:pNH_3$ ratios greater than approximately 0.8. Addition of calcium chloride or acid to UW85 culture filtrate to lower the $pCa^{2+}:pNH_3$ ratio reduces zoospore lysis. Uninoculated growth medium becomes lytic when supplemented with ammonium chloride and base to create a $pCa^{2+}:pNH_3$ ratio greater than 0.8. The relevance of zoospore lysis by UW85 to plant protection is not yet known.

P0-72

DELETERIOUS RHIZOBACTERIA FOR BIOCONTROL OF WEED SEEDS AND
SEEDLINGS IN SOIL

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Over 500 bacterial isolates from weed seedling rhizospheres have been screened for ability to inhibit weed seed germination and decrease seedling growth. In vitro screening revealed that 56, 62, 49, 48, and 64% of rhizobacterial isolates significantly inhibited seedling development of their respective hosts: velvetleaf, morningglory, cocklebur, pigweed, and jimsonweed. Effects of these deleterious rhizobacteria (DRB) were root and shoot growth inhibition, root necrosis and leaf chlorosis. Examination of seedling root surfaces by scanning electron microscopy revealed characteristic colonization patterns by DRB. Additional bioassays indicated that DRB may cause colonization of the weed seedling root. Soil inoculation with selected DRB in greenhouse and field studies resulted in decreased velvetleaf seed viability, reduced seedling emergence, decreased seedling vigor, and severely damaged root systems. Velvetleaf seed/seedling exudates were found to specifically elicit chemotactic responses by DRB. Development of rhizobacteria deleterious to weed seeds/seedlings holds potential as a biocontrol method.

P0-73

EVALUATION OF THE EFFECTS OF BIOCONTROL AGENTS ON
MYCORRHIZAL FUNGI

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Biocontrol agents (BCA) to control soilborne root pathogens are being evaluated for effects on non-target mycorrhizal fungi that are symbiotic with the roots of most plants. Evaluation criteria include effects of the BCA on spore germination, root colonization dynamics, development of external hyphae or rhizomorphs, plant growth enhancement under nutrient stress, changes in root respiration, and in vitro antagonism. Ectomycorrhizal, ericoid, and VA mycorrhizal fungi on Douglas-fir, cranberry, and onion or cucumber, respectively, are being challenged by species of Trichoderma, Gliocladium, Talaromyces, Pseudomonas, Bacillus, Agrobacterium, Enterobacter, Alcaligenes, Streptomyces, and Serratia. Preliminary results indicate that most of the bacterial or fungal BCA are compatible with mycorrhizal fungi.

P0-74

**AN IMPROVED, IN VITRO TECHNIQUE FOR RAPIDLY ASSAYING
RHIZOSPHERE BACTERIA FOR THE PRODUCTION OF
COMPOUNDS INHIBITORY TO RHIZOCTONIA SOLANI AND
GAEUMANNOMYCES GRAMINIS VAR TRITICI.**

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An improved, in vitro method for efficiently selecting rhizosphere bacteria which produce antibiotics is described. Bacterial colonies which have lost the ability to produce antibiotics after chemical or site directed mutagenesis can also be quickly identified using this technique. Current methods test 1 to 4 organisms per 9 cm petri plate and take up to 10 days. Our assay allows 12 bacterial isolates per petri plate to be appraised for antifungal activity and takes 4 days. Fungal pathogens are grown in pure culture on pieces of moistened, pulverized rice hulls. Bacterial isolates are transferred to petri dishes containing nutrient media and the surface of the medium is sprinkled with rice hulls colonized by the fungal pathogen. Zones of inhibited fungal growth around bacterial colonies are distinct after incubating plates at 25° C. for approximately 4 days, depending on the test medium and fungus used. This technique was used to measure the impact of tillage and crop rotation on the proportion of wheat rhizosphere microbes antagonistic to *Rhizoctonia solani* and *Gaeumannomyces graminis* var *tritici*. Tillage rarely significantly influenced the percentage of antagonistic "total" aerobic bacteria (TAB), pseudomonads (PS), or heat resistant organisms. A pasture-wheat rotation fostered higher percentages of antagonistic TAB and PS than did continuous wheat though the populations of TAB and PS were higher under continuous wheat. The assay medium and test fungus significantly influenced the percentage of isolates deemed antagonistic.

P0-75

**SICK PATHOGENS MAKE POOR PESTS in BIOLOGICAL CONTROL OF CORN
DISEASES.**

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Soil-borne mycopathogenic fungi, Melanospora damnosa, Nectria gliocladioides, and Sphaeronaemella helvellae infect fungi that are pathogens of corn (Zea mays L.). Different single-ascospore cultures of M. damnosa and S. helvellae, in dual culture with a host fungus, displayed a range of reactions from non-mycopathogenic to biotrophic to necrotrophic. Treatment of corn kernels with spore suspensions of each mycopathogen increased the vigor (dry weight) of the seedlings and reduced colonization of the germinated kernels by fungi when compared with water treatment. These results were consistent in agar medium, controlled seed germinator, greenhouse sand bench, and in the field. Selection of single-ascospore cultures of S. helvellae for greater mycopathogenicity to Cochliobolus carbonum in dual culture, resulted in a highly significant reduction in colonization of germinated kernels by C. carbonum when compared with non-mycopathogenic cultures, when both fungi were used for coating kernels. Treatment of corn foliage with spore suspension of mycopathogens during the growing season increased yield of inbred corn lines when compared with water treatment. Some corn genotypes interacted with mycopathogens resulting in even greater yields.

P0-76 FUNGAL ANTAGONISTS OF PYTHIUM ULTIMUM FROM A SUPPRESSIVE
SPHAGNUM PEAT

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A commercial Sphagnum peat was shown to be suppressive to damping-off and root rot of cucumber caused by Pythium ultimum. The suppressiveness was greatly reduced by heat treatment at 60°C or by addition of benomyl to the peat whereas several applied antibiotics had no effect on the suppressiveness. In order to investigate the possible causal agents of the suppressiveness, several microorganisms were isolated from the peat and later added to peat that had been heat treated at 100°C. Of the microorganisms tested, Trichoderma harzianum, Geomyces pannorum, and a mixture of Penicillium spp. and Aspergillus spp. were able to suppress Pythium attack of cucumber seedlings whereas actinomycetes and other bacteria had no effect. Gliocladium virens inhibited plant growth in the heat treated peat. T. harzianum and G. virens were then tested in an untreated conducive peat, and both were able to control Pythium. The results indicate that fungal antagonists are responsible for the suppression of Pythium ultimum in the peat. Several antagonists have been isolated, but the role of each individual antagonist still needs to be determined.

P0-80 SIDEROPHORE-PRODUCING BACTERIA ISOLATED FROM ROOTS OF IRON
EFFICIENT AND INEFFICIENT GRASSES

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Virtually all aerobic and facultatively anaerobic soil bacteria produce siderophores as a means of scavenging iron under iron-deficient conditions. It has been suggested that siderophores produced by rhizosphere bacteria may facilitate iron acquisition by plants by increasing the availability of iron in the root environment. In a greenhouse experiment, we have examined the populations of siderophore-producing bacteria (SPB) colonizing the roots of St. Augustinegrass, a turfgrass which commonly exhibits iron chlorosis, and bermudagrass, a turfgrass which resists iron chlorosis. Total counts of aerobic, heterotrophic bacteria cultured on Chrome Azurol S (CAS) agar were 10-fold greater in homogenates of washed St. Augustinegrass roots as compared to bermudagrass roots. Numbers of SPB, evidenced by production of an orange halo on CAS agar, were similar for the two grasses. As a result, the proportion of SPB on bermudagrass roots was greater. Similar results were obtained with grasses grown in sand mixed with 2 mg Fe (ferrihydrite) kg⁻¹ or 10 mg Fe kg⁻¹. Different species of SPB were isolated from the two grasses, though most were members of the genus *Pseudomonas*.

P0-81

PRODUCTION OF SIDEROPHORE-LIKE IRON CHELATORS BY ERICOID AND ECTOMYCORRHIZAL FUNGI

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The Schwyn and Neilands chrome azurol S (CAS) universal siderophore assay (Anal Biochem 160:47) was adapted to screen for production of iron chelators by ericoid and ectomycorrhizal fungi. Two ericoid mycorrhizal fungi, Hymenoscyphus ericae and Oidiodendron griseum, and the ectomycorrhizal fungus Rhizopogon vinicolor produced rapid extensive color changes characteristic of iron removal from the CAS-iron(III)-detergent complex. Cenococcum geophilum and Hebeloma crustuliniforme produced weaker color reactions, while Laccaria laccata produced no detectable reaction and failed to grow on the medium. Correspondence between presumptive siderophore production and ability of these mycorrhizal fungi to inhibit growth of a soil actinomycete further supports the concept of iron chelation as one mechanism of competition within the mycorrhizosphere.

P0-82

METABOLISM of BRADYRHIZOBIUM SIDEROPHORES BY SOYBEAN CELLSHarry Calvert,¹ Sarah Marsh,¹ and Minocher Reporter^{1,2}¹C. F. Kettering Research Laboratory, Yellow Springs, OH 45387, and ²NSI Services Technology, Inc., 200 S.W. 35th Street, Corvallis, OR 97333

Iron metabolism is important in symbiosis. Nodule bacteroids require the iron for making protoheme IX, which is exported into the plant cell cytoplasm to form leghemoglobin, nitrogenase ferredoxin iron-sulfur centers, and a number of cytochromes. We previously showed that Bradyrhizobium japonicum, under iron stress, excreted yellow-green fluorescent peptide (GYP) into the growth medium. The peptide bound one Fe III per mole. It also loosely bound Fe II and other transition metals with the binding proportionately quenching the peptide fluorescence. When added to soybean cell culture medium, the peptide was metabolized, as shown by: (a) loss of fluorescence; (b) loss in amount of peptide precipitated by antiserum to the GYP and uptake of ⁵⁵Fe-peptide into the plant cells. Autoradiography of the ⁵⁵Fe-label taken up by the plant cells indicated only complexed iron (EDTA or GYP) could be localized into cells. The label was predominantly in older cells. The peptide may not be available to young cells from outside, but if it is excreted by bacteroids from within the cells, then it could be used by both plant cells and bacteroids for iron mobilization.

P0-83

GENETIC IMPROVEMENT OF SIDEROPHORE PRODUCTION AIMED AT ENHANCING BIOCONTROL IN PSEUDOMONAS STRAINS.

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Certain soil fluorescent Pseudomonads are effective in promoting plant growth by inhibiting deleterious bacteria and fungi. Iron deprivation due to the secretion of iron-binding ligands (siderophores) may be a major factor in the disease suppression ability of these Pseudomonas spp. Strains isolated from the roots of sugarbeet were screened for high affinity siderophore system and for aggressive colonizing ability. Tn5 mutants in the siderophore biosynthetic uptake and regulatory functions were induced. Complementation of the biosynthetic mutants of Pseudomonas sp. strain M114, by a pLAFR1-based gene bank, resulted in the isolation of five overlapping cosmid clones. The transcriptional regulation of the genes encoding this siderophore-mediated iron uptake system was studied by constructing Fe^{3+} -regulated promoter-lacZ fusions. They were exploited to screen for mutants where the regulation imposed by iron on the expression of the siderophore genes was broken. One mutant produced siderophores when iron was present in the growth medium. Further characterization revealed some deregulation of the expression of the outer membrane receptor proteins. Improved inhibition of bacterial and fungal organisms was observed on high iron media when compared with the wild-type.

P0-84

RHIZOBACTIN, A STRUCTURALLY NOVEL SIDEROPHORE BIOCHEMICALLY RELATED TO THE OPINES

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The recent structure elucidation of rhizobactin(RB), N^2 -[2-[D-(1-carboxyethyl)-amino]ethyl]- N^6 -L-(3-carboxy-3-hydroxy-1-oxopropyl)-L-lysine, from *Rhizobium meliloti* DM4 disclosed a third chemical class of microbial siderophores (Gr. *sidero* = iron; *phore* = bearer); the ethylenediamine ligand was also unprecedented as a natural product. Yet determination of the absolute configuration and proof of the entire structure awaited total synthesis, which is now reported. Several lines of evidence demonstrate that this siderophore is related in structure and activity to other N^2 -substituted amino acids termed opines. RB can be considered as a "bis-opine," produced by reductive amination of the opine D-strombine, i.e., N^2 -[2-[D-(1-carboxyethyl)-glycine, with N^6 -L-(3-carboxy-3-hydroxy-1-oxopropyl)-L-lysine, for it fulfills the $D^{\alpha\text{la}}, L^{\text{amino acid}}$ rule of pyruvic acid derived opines. Opine biosynthesis and utilization is strain-specific, according to the particular virulence plasmid present. Likewise, RB biosynthesis and utilization is strain-specific. Moreover the genetic inference that agrobacteria cannot produce opines is equivocal, while the production of RB by iron-deprived *Rh. meliloti* DM4 constitutes evidence to the contrary. That rhizobactin, opines and phytosiderophores display similar selectivities toward metal ions carries provocative implications for metal ion metabolism in the rhizosphere. Current work on the bioinorganic mode of action of this important siderophore will be presented.

P0-90 IN VITRO STUDIES ON THE INTERACTIONS OF AGROBACTERIUM spp.
AND PSEUDOMONAS spp. ISOLATED FROM OPINE ENVIRONMENTS.

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The growth parameters of octopine catabolizing bacteria A. tumefaciens, strains B6 and 15955, and P. fluorescens, strains B99A and E175D, have been examined under octopine limitation in chemostats. The two pseudomonads were isolated from crown galls. The maximum specific growth rates (μ_{max}) of all four strains with octopine as substrate were comparable. The substrate affinities (K_s) indicated that octopine was utilized much less efficiently as a substrate than glutamate. Yield coefficients revealed that octopine could be utilized much more efficiently when supplied as the source of limiting nitrogen than as the sole source of limiting carbon. Monod curves generated with these parameters produced models of the growth and competition of these strains. A. tumefaciens strain B6 was notable for its poor competitive potential when growing on octopine. Dual culture chemostat runs of pseudomonads + agrobacteria were always dominated by the pseudomonad sometimes in contradiction of the models. Although both pseudomonads are fluorescent, siderophores are not considered the prime antagonistic agent.

P0-91 COLONIZATION OF WHEAT ROOTS BY PSEUDOMONAS FLUORESCENS: SEM OBSERVATIONS AND BIOCHEMICAL ANALYSIS

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The interaction of selected fluorescent *Pseudomonas* spp. with the roots of wheat seedlings was studied using a gnotobiotic system. Fingerprint characterization at strain level by two-dimensional (2D) protein analysis aided to inventory our *Pseudomonas* collection. Scanning electron microscopy (SEM) of colonized root surfaces revealed the presence of rod-like structures connecting the bacterial cells with the root. This observation, initially made with strain OE 28.3, was extended to several other strains, including NRRL B-15132 (2-79). A correlation was found between the occurrence of these connections at the root surface and the constitutive production of a major 33 kDa outer membrane protein, displaying pI heterogeneity among strains. Comparative 2D analysis of root extracts from inoculated and axenic plants revealed the presence of a single additional acidic protein on washed roots from inoculated plants. It proved to be the aforementioned bacterial 33 kDa protein. Apparently, this protein remained firmly attached to the root system only when possessing a specific pI value. The above data suggest that the 33 kDa protein may be involved in generating the rod-like structures, that possibly play a role in root attachment.

P0-92

MUTATIONAL CHANGES IN THE O-ANTIGENIC SIDE CHAIN OF
THE LIPOPOLYSACCHARIDES OF PSEUDOMONAS spp. AFFECT
COLONIZATION OF- BUT NOT ADHESION TO- POTATO ROOTS.

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Some bacteriophage resistant mutants of the root-colonizing Pseudomonas strains WCS358 and WCS374 lack the O-antigenic side chain of the lipopolysaccharide (LPS). Colonization experiments showed that inoculation of the roots with one of these mutant strains resulted in colonization of the deeper root parts in less than 50% of replicate plants, while the wild type strains colonized the whole root system in more than 90% of the replicate plants. The cell surface charge and hydrophobicity of these mutant strains were shown to differ from those of the wild types. This made us look at the interaction of these strains with the potato root surface. However, the firm adhesion of mutant and parent strains to sterile roots did not differ significantly. These results indicate that the presence of O-antigenic side chains of the LPS is involved in optimal potato root colonization by Pseudomonas spp., but that the O-antigen structure does not exert this effect at the stage of firm interaction to the plant root surface.



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